Probe

Newsletter for the USDA Plant Genome Research Program

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USDA's Office of Agricultural Biotechnology

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Office of Agricultural Biotechnology, USDA
Washington, D.C.

Can bioengineered organisms of agricultural importance be released safely into the environment and the marketplace? Will consumers' desires for labeling of biotech products impact on the proposed Food and Drug Administration's policies? What will be the impact of BST on the dairy industry? Will biotechnology technologies help revitalize rural America? Can this new technology be useful in preventing and detecting food safety problems? These and other questions are routinely being addressed by the USDA Office of Agricultural Biotechnology.

The Office of Agricultural Biotechnology (OAB) was established in 1986 by a Secretary's Memorandum 1020-27. Its role is to coordinate the development of consistent biotechnology policies and procedures within USDA.

Current OAB functions include:

- Under a Presidential Initiative, OAB is the USDA action office for a multi-year Federal initiative on biotechnology research.
- OAB staffs a Federal advisory committee, the Agricultural Biotechnology Research Advisory Committee (ABRAC). This Committee provides a public forum for issues in agricultural biotechnology.
- OAB staffs the Committee on Biotechnology in Agriculture (CBA), composed of six USDA Agency Administrators and two Assistant Secretaries, and the Biotechnology Council, composed of senior agency staff.
- OAB has provided leadership for the development of
  -biotechnology guidelines for agricultural research;
  -scientific exchanges involving biotechnology;
  -environmental assessments for transgenic fish;
  -performance standards for research with transgenic fish and shellfish;
  -advice when requested by regulatory agencies;
  -a biotechnology consumer information plan for USDA;
  -coordinated responses on regulatory and research issues to other Departments;
  -international conferences/workshops on animal and plant biotechnology, including three international conferences on "The Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms";
  -staff papers and speeches for the Office of the Secretary.

Since the implementation of the January 31, 1992, Presidential Initiative on Biotechnology Research, OAB has been the action office for USDA. Twelve Federal agencies participate in the activity. Their efforts on the biotechnology crosscut are coordinated by the Biotechnology Research Subcommittee (BRS) of the Committee on Fundamental Science and Engineering Research and Development of the National Science and Technology Council. As a participant, OAB has collected research program and budget data from the Agricultural Research

Service, Cooperative State Research Service, Economic Research Service and the Forest Service and eight non-USDA agencies which are involved in agriculturally related programs and assembled data into a consistent format for reporting.

The ABRAC consists of 15 experts from academia, industry, government, and public interest groups with knowledge and experience in one or more of the following areas: recombinant DNA research in plants, animals, and microbes; ecology and environmental science; agricultural production practices; biological containment and field release; applicable laws and regulations; standards of professional conduct and practice; public attitudes; public health/epidemiology; and occupational health and ethics. Fifteen ABRAC members have been recently appointed by the Secretary of Agriculture.

The purpose of ABRAC is to advise the Department, through the Assistant Secretary for Science and Education, with respect to policies, programs, operations, and activities associated with questions of biosafety, the development of guidelines and performance standards for research with genetically modified organisms, and, in response to a specific request, the development of recommendation for the food safety evaluation of transgenic livestock.

The most important issue that will be addressed by the new Committee in the coming months will be to complete the development of performance standards for outdoor research with genetically modified fish and shellfish. Other important issues may include: management of resistance to biopesticides in crop plants; production of pharmaceuticals in plants and animals; use and effects of synthetic sequences in organisms of agricultural importance; risk management and risk communications; and public attitudes, perceptions, and acceptance of genetically engineered products.

In addition to the scientific aspects of modern biotechnology, OAB clearly recognizes the public relations dimension of biotechnology. A well-informed public is better able to participate in the decision-making process about biotechnology. Toward this end, OAB shares information with the media, participates in public affairs activities around the country, and contributes articles on biotechnology to the general as well as the scientific press. The Office publishes a monthly newsletter, *Biotechnology Notes*. The newsletter highlights activities on biotechnology issues at USDA and in the private sector. The dissemination of the newsletter is via mail, through USDA's Computerized Information Delivery System, and on Internet through the National Agricultural Library's Gopher; provided by the Biotechnology Information Center. From time to time the Office has sponsored national and international conferences and workshops on biotechnology topics.

The international and trade implications of agricultural biotechnology are of growing concern to many U.S. economic planners and policymakers. On the international front, OAB is involved in studying research and technology transfer programs in competing nations; promoting international consensus on the scientific principles that underlie the environmental and human safety of agricultural biotechnology; and working with the U.S. Trade Representative, the Food and Drug Administration and the Foreign Agricultural Service to provide information to trading partners regarding U.S. food safety procedures.

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Michigan State Univ.

Dr. Walter Reid
World Resources Institute

Dr. Paul Thompson
Center for Biotechnology Policy and Ethics
Texas A&M Univ.

Minutes from ABRAC meeting are published and available from the OAB on request. (703) 235-4419

Needless to say, the OAB program is a very dynamic office that maintains timely responsiveness to the ever-changing biotechnology scene.

All the above activities are done with a small core of permanent staff, but OAB Director Dr. Alvin Young says that "much of my staff work is done by individuals on temporary assignment to OAB from other USDA agencies." Specialists in agricultural research, extension/technology transfer, regulations, environmental impact, economics, public relations, and international affairs have been supplied to OAB by cooperating agencies. At the completion of their assignments, says Young, "these individuals take their new biotechnology knowledge and experience with them back to their agencies and everyone benefits."

For additional information or to request OAB published materials, contact the OAB at (703) 235-4419. ◆

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National Agricultural Library

Summary of the 1993 Plant Genome Awards

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Washington, DC 20250

In 1993, Congress appropriated $97.5 million for the National Research Initiative Competitive Grants Program (NRICGP), of which $12.1 million was made available for plant genome research. The Plants Division of the NRICGP in USDA's Cooperative State Research Service administers the plant genome grants. In addition to the NRICGP allocation, $3.67 million was appropriated to USDA's Agricultural Research Service (ARS) for program management, setting targets for genome mapping research, and database development for agriculturally important plants. After program administration costs, the net amount available for all plant genome research totalled $15.1 million: $3.0 million for the ARS and $12.1 million for the NRICGP. Mission-oriented research proposals that address the goal of improving agronomic qualities through genomic research were submitted to the NRICGP by scientists from the research community. Each proposal was peer-reviewed by experts in the area of genomic research and was

Continued page 7

Table 1

SPECIES REPRESENTED BY PLANT GENOME AWARDS 1993

SPECIES	NUMBER OF AWARDS	DOLLAR AMOUNT
Agrobacterium	2	250,000
Alfalfa	2	220,000
Apple	1	276,000
Arabidopsis	8	539,000
Barley	5	961,000
Bean	2	370,000
Blueberry	1	118,422
Brassica	4	262,000
Cotton	2	299,013
Douglas Fir	1	223,000
Lettuce	1	200,000
Maize	20	2,405,700
Millet	1	120,000
Mungbean	1	115,000
Oats	2	300,000
Peach	1	200,000
Peanut	2	262,000
Petunia	2	211,000
Pine	3	453,000
Poplar	1	120,000
Potato	2	190,000
Rice	3	570,000
Sorghum	4	297,200
Soybean	3	500,000
Tobacco	11	1,392,400
Tomato	7	846,900
Wheat	6	1,145,200
Wild Rice	1	40,000
Totals	91	12,126,238

ARS G.A. Smith, J. Miksche

* Summations of award numbers and dollar amounts do not sum to the actual values presented because some of the awards utilized more than one species.

Table 2

GENE SYSTEMS OF TRAITS, NRI PLANT GENOME GRANTS, 1993	
AC/DC transposons	Anthocyanin biosynthesis
AC/Ds mutagenesis and integration	Aphid resistance
Alcohol dehydrogenase	Blight resistance
Amylase activation	Endodormancy chilling requirement
Apomixis	Ethylene biosynthesis
Bacterial blight resistance	Fertility
Ca+ Modulated leaf receptor protein	Fiber quality
Cytokinin response	Fruit quality
Disease resistance	Hessian fly resistance
Drought tolerance	Inflorescence development
Floral homeotic genes and sterility	Kernel starch
Flowering	Kernel sucrose metabolism
Gene targeting for excision of foreign DNA	Leaf epidermal growth
Lipid desaturation	Mildew resistance
mRNA stability	Mitochondria protein synthesis
Phytochrome A mRNA degradation	Nematode resistance
Plasmid directed conjugation	Nodulation and N fixation
Polyamines and stress tolerance	Phaseolin and seed protein
QTLs for wood quality	Photorespiration
QTLs for yield	Plastid light response
Ribosomal protein synthesis	Rust resistance
Rust resistance	Seed oil synthesis
Seed maturation	Self incompatibility
Stable transformation	Ubiquitin ligation
Starch synthesis	Vigor and plant morphology
Targeted DNA integration	Virulence genes
Trichomes and insect resistance	Virus resistance
T-DNA transport and integration	Wood specific gravity

Table 3

GENETIC PHENOMENA NRI PLANT GENOME GRANTS 1993

DESCRIPTION	SPECIES
Amylase activation & repression	Barley, Maize
Annual growth rate	Douglas fir
Anthocyanin biosynthesis	Maize
Apomixis, asexual reproduction	Pearl millet
Cell growth	Tobacco
Chromosome recombination	Wheat
Chromosome sorting libraries	Tomato
Cloning disease resistance genes	Rice
Cytokinin response	*Arabidopsis*
Developmental regulation	Tomato, Tobacco
Drought tolerance	Grain sorghum
Edthylene biosynthesis	Tomato
Endodormancy & chilling requirement	Blueberry
Gene effects on chromatin structure	Maize
Gene tagging for insect resistance	Maize
Genetic engineered sterility	Poplar
Gene/chromosome identification	Wheat
Homologous recombination	*Arabidopsis*
Homozygous deletion stocks	Wheat
Hormonal control of seed maturation	Tobacco
Inflouresence development, flowering	Maize, Tobacco
Lipid desaturation	Peanut
Mapping cold, disease resistance	Peach
mRNA stability in dicots	Tobacco
Mutation via transposable elements	Tomato
Nodulation & N fixation	Bean, Medicago
Nuclear targeting of DNA	Agrobacterium
Nuclear & organelle DNA variation	Barley
Nuclear-plastid communication	Tobacco
Photorespiration, nitrogen assimilation	*Arabidopsis*
Phytochrome A, mRNA degradation	Oats
Plasmid directed conjugation	Agrobacterium
Phytochrome gene control	Tomato, Rice, Sorghum
Repeat induced gene silencing	*Arabidopsis*
RNA editing	Petunia
Seed oil synthesis	Maize, Brassica
Self incompatibility	Brassica
Sequence repeats, microsatellites	Soybean
Site directed mutagenesis	Maize
Spatial organization of genome	Maize, Sorghum
Starch metabolism & transport	Maize
Tandem repeat sequences	Rice
Targeted DNA integration	Tobacco
Transformation disease resistance	Potato
Transformation drought tolerance	Pine
Transformation insect resistance	Tomato
Transformation virus resistance	Peanut
Transposable elements	Maize
Transposon tagging	Maize
T-DNA transfer, and transport	Tobacco, Maize
Ubiquitin activating enzymes	*Arabidopsis*
Wound inducible insect resistance	Tobacco

Awards--Continued from page 4

judged on its scientific and technical merit, qualifications of proposed personnel, and relevance to sustainability and stated research objectives in the solicitation for proposals.

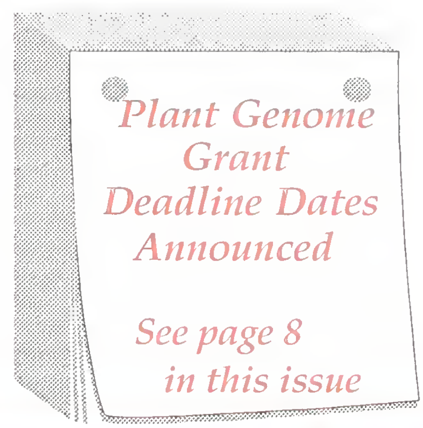
In 1993, the majority of plant genome funds supported awards made in two programs in the NRICGP Plant Division: the Plant Genome Program and the Plant Genetic Mechanisms Program. A small portion of funds also supported genome-related research in other progams of NRICGP. Research projects being supported in FY 1993 are summarized in the accompanying tables.

Ninety-one awards were made to scientists from 34 States (See article, "The Class of 1993 Plant Genome Grant Recipients" in this issue).

Nineteen agronomic, horticultural, and forest tree species are undergoing genetic and physical mapping procedures (Table 1). Fifty-six genes and gene systems are being studied (Table 2), as well as genetic phenomena in various plant species (Table 3). Many new molecular techniques are being pursued (Table 4). The data were supplied by the NRICGP staff, and compiled into tables by Dr. G.S. Smith and Dr. J.P. Miksche in ARS. ◆

Table 4

TECHNIQUE DEVELOPMENT NRI PLANT GENOME GRANTS, 1993
Antibody synthesis for proline
Biolistic transformation with high molecular weight DNA
Chromosome painting
Construction and insertion of chimeric gene
Develop target probes for fly resistance
DNA vector synthesis
Functional cloning of disease resistant genes
Identification of effective sterility genes
Isolate nuclear skeletal structures
Map based cloning system for Rice
Megabase DNA isolation
Molecular probe and reagent development
Regeneration after transformation for Pine
Substrate features required for enzyme activation
Transfer teosinte chromosomes to maize
Transformation system for Peanut
Transposon tagging
Wound induced transformation



Off the Wire

RBNET (Electronic Mail Network for Rice Biotechnologists)

With support from the Rockefeller Foundation, an electronic mail network has been established to increase communication among the international rice biotechnology community. This network (RBNET) is being managed by Professor Verma at the Ohio State Biotechnology Center, 1060 Carmack Road, Columbus, Ohio 43210 USA. To address all users of the network you may send a message to: rbnet@magnus.acs.ohio-state.edu. To add your name to the RBNET mailing list, please send your E-Mail address to: dverma@magnus.acs.ohio-state.edu.

For those colleagues in countries where electronic mail service is not currently available, attempts are being made to deliver a message by fax. If any one of you are in this situation and wish to communicate to the RBNET users, you may fax your message to the attention of Professor Verma at (614) 292-5379 and your message will be posted on the RBNET for distribution.◆

1995 NRI Grant Deadlines

Postmarked Dates	Program Codes	Program Areas	Contacts (202)
November 14, 1994	31.0 52.1 52.2	Improving Human Nutrition for Optimal Health Plant Genome Plant Genetic Mechanisms	205-0250 401-1901 401-5042
November 21, 1994	22.1	Plant Responses to the Environment	401-4871
December 5, 1994	51.1 54.1	Plant Pathology Photosynthesis and Respiration	401-4310 401-6030
December 12, 1994	25.0	Soils and Soil Biology	401-4082
December 19, 1994	26.0 51.5	Water Resources Assessment and Protection Biological Control Research	401-4504 401-5114
January 9, 1995	51.2 51.3 51.4 44.0	Entomology Nematology Weed Science Sustaining Animal Health and Well-Being	401-5114 401-5114 401-4310 401-6303
January 17, 1995	71.1 71.2 72.0 41.0	Food Characterization/Process/Product Research Non-Food Characterization/Process/Product Research Biofuels Research Enhancing Animal Reproductive Efficiency	401-1952 401-1952 401-1952 401-6234
January 23, 1995	23.0 53.0	Forest/Range/Crop/Aquatic Ecosystems Plant Growth and Development	401-4082 401-5042
January 30, 1995	32.0 51.6 100	Ensuring Food Safety Assessing Pest Control Strategies Agricultural Systems	401-4399 401-5114 401-1901 401-6303
February 6, 1995	54.2 61.0 62.0	Nitrogen Fixation/Nitrogen Metabolism Markets and Trade Rural Development	401-6030 401-3487 401-3487
February 13, 1995	73.0	Improved Utilization of Wood and Wood Fiber	401-4871
February 21, 1995	42.0 43.0	Improving Animal Growth and Development Identifying Animal Genetic Mechanisms and Gene Mapping	205-0250 401-4399
February 27, 1995	80.1 80.2 80.3	Research Career Enhancement Awards Equipment Grants Seed Grants	401-6234 401-6234 401-6234

The 1995 program solicitation and application kit will be available in September. Please note that potential applicants who are on the competitive research grants mailing list, who sent in applications in fiscal year 1994, or who recently requested placement on the list for fiscal year 1995 will automatically receive copies of the program solicitation and the NRICGP application kit. All others may request copies from:

National Research Initiative Competitive Grants Program (NRICGP)
c/o Proposal Services Branch
AMD/CSRS/USDA
AG Box 2245
Washington, DC 20250-2245
Telephone: (202) 401-5048

For more information about the Plant Genome Grants Program, contact:

Dr. Ed Kaleikau
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New Capabilities and Connections for the Plant Genome Database

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The Plant Genome Database has been considerably augmented in the past few months. A number of new connections and capabilities have been added to the World Wide Web (WWW) and gopher interfaces, and a text-based Lynx client has been added to provide access to those with slower Internet connections and/or low resolution graphics. The following is a summary list of recent changes to the Plant Genome Database:

1) WWW Interface to ACEDB Databases, 2) New Query Capabilities for Plant Genome Data, 3) Links to External Data (Agricola, GenBank, Swiss-Prot, Images), 4) New Gopher Services, 5) Telnet Access to WWW Lynx Client, 6) Collaborator's Page summarizes Collaborator's Services, 7) New Draft for Plant Genome III Meeting, 8) New Solanaceae Database Shows Syntenic Relationships

WWW Interface to ACEDB Databases

The Plant Genome Database Project is now offering a collection of ACEDB-based databases for both plant and non-plant genomes. The databases have been installed behind the "Moulon" ACEDB-WWW server and can be browsed and queried interactively. The various databases are listed below:

Plants

- AAtDB—*Arabidopsis*
- SoyBase—soybeans
- RiceGenes—rice
- MaizeDB—maize
- GrainGenes—wheat, barley, rye, relatives
- TreeGenes—forest trees
- SolGenes—Solanaceae

Other organisms

- ACeDB—C. elegans
- AceMap—Human Chromosome X
- FlyDB—Drosophila melanogaster
- MycDB—mycobacteria
- AGsDB—A Genus species Database

New Query Capabilities for Plant Genome Data

The ACEDB-based databases can be queried using the ACEDB query language, query-by-example (QBE) and a query-building (QB) tool. The ACEDB query language requires an understanding of syntax, but QBE and QB do not. In addition, all the data from the ACEDB databases has been indexed for searches using WAIS. Finally, data can be searched using agrep—a tool which allows you to make approximate matches (aka, fuzzy searching).

Links to External Data: AGRICOLA, GenBank, SwissProt, Images

HTML links are increasingly being used to connect plant genome data with data from external sources, for example AGRICOLA, GenBank, and SwissProt. This allows us to take advantage of the division of labor between databases specializing in different tasks. For example, the receptor kinase sequence object from the Arabidopsis database AAtDB contains a link to GenBank labeled "M80238". Clicking on it will retrieve a GenBank record from a computer at National Institutes of Health.

New Gopher Services

The plant genome information on the agricultural genome Gopher has been reorganized. It can be used to access the same information that is presented by the WWW interface to the genome databases, both plant and non-plant.

Telnet Access to WWW Lynx Client

Users who are restricted to VT100 or VT200 terminals or emulators can access the WWW services using Lynx, a text-only client (see below for access details). The client is set up so that only NAL data (and data from a few other sources) is available—it cannot be used to surf the Internet (sorry).

Collaborator's Page Summarizes Collaborator's Services

A page has been set up with links to resources maintained independently by the plant genome collaborators. The services include ftp archives, gophers, and access to databases.

New Draft for Plant Genome III Meeting

There is a new version of a draft describing the next plant genome meeting in San Diego in mid-January 1995 (gopher and WWW).

New Solanaceae Database Shows Syntenic Relationships

SolGenes, a database for pepper, tomato, and potato, now shows the syntenic relationships between chromosomes from these members of the Solanaceae. The information is presented as an on-line image of a genetic map. The image responds to mouse clicks enabling one to find out more about a particular locus by clicking on it, then selecting "Show as text." Figure 1 is an example comparing chromosome 9 of tomato and potato. Access to these graphically depicted relationships from the agricultural genome home page is via the following sequential menu selections (WWW Interface to ACEDB Data, Browse Solgenes, and Multimap).

Ways to Access the Agricultural Genome Databases

WWW:
http://probe.nalusda.gov:8000/

Gopher:
gopher probe.nalusda.gov

Lynx:
telnet probe.nalusda.gov
login: lynx
password: (none-hit return)

Anonymous FTP:
probe.nalusda.gov

Plant Genome III Meeting Announcement

The Science and Technology Coordinating Committee of the USDA Plant Genome Research Program will hold an open meeting on Monday, January 16, 1995 from 7:30 pm to 10:00 pm. The Committee will be reviewing the progress of the Plant Genome Research Program. All Plant Genome III attendees are invited to participate and express their views.

Announcing

Plant Genome III

Town & Country Hotel, San Diego, CA

Comparative Genetic Mapping
Isolation and Transformation of Agriculturally Important Genes
Instrumentation/Technology
Applications of cDNA Research
Chromosome Structure
ALFPs/QLTs/Metabolic Pathways

$300 Advance Registration up to December 1, 1994
$350 after December 1, 1994 and on-site
$100 Student (Pre-Ph.D) registration
(Requires a letter of certification from department chairperson.)

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Plant Genome II Conference Report

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Plant Genome II featured applications of genome mapping and analysis to solve existing problems and uncover answers to fundamental questions relating to the plant genome and its evolution. The meeting was held in San Diego, California from Janaury 24 to January 27, 1994, and attracted 553 participants from 22 countries.

According to Steven Oliver, University of Manchester, Manchester, United Kingdom, we are entering a stage where the taxonomy of gene function will be essential in efficiently identifying new genes. This new stage will require a multidisciplinary approach encouraging the collaboration of physiologists, geneticists, biochemists, and plant breeders. He defined this era as a new voyage of the *Beagle*.

The message was reinforced by Dr. James Cook, USDA, ARS and also Senior Scientist at CSRS, who pointed out that now is the time to bring plant breeders together with molecular biologists to conduct a gene hunt for agronomically important genes.

New Insights

Understanding plant genome structure and organization can lead to interesting and relevant discoveries, as highlighted by Dr. Richard Flavell, Director, John Innes Institute, Norwich, United Kingdom. According to Flavell, understanding the role of epigenetic regulation, gene order, and *in situ* homology sequence searching will ultimately help in the practical application of biotechnology. Plants have had to defend themselves from foreign DNA over the millennia and as a result have developed strategies -- including gene silencing -- to cope with transposon selection pressures. The plant's ancient art of anti-sense technology may take advantage of gene location. Gene location would determine epigenetic DNA methylation events, which in turn would regulate gene expression.

In all, Flavell points out, concerted evolution in the long term helps to maintain high levels of conservation across the chromosome both in terms of sequence and gene order or synteny.

Thomas Bureau, University of Georgia, detected evidence of ancient transposon and retrotransposon events. Extensive sequence similarity searches were performed on the GenBank and EMBL nucleotide sequence databases. These "database mining" experiments have identified over 100 normal plant gene sequences showing evidence for a member of either the "Tourist" or "Stowaway" family of transposons. The location of several elements corresponds to previously reported *cis*-acting regulatory elements. Significantly, a "Tourist" element was found to serve as the promoter for the maize auxin-binding protein (*abp1*). The first plant retrotransposon, *Bs1*, was found to contain a cellular gene fragment; this provides the first evidence for transduction by a retrotransposon in plants.

Progress in Rice

The Japanese Rice Genome Program reported significant advances. Dr. Nori Kurata, NIAR/STAFF, Japan, described a genetic map with 1,400 RFLP and RAPD markers. Over 7,500 clones from callus tissue at different developmental stages have been sequenced. Of those sequenced 1,800 are clones of known function. Dr. Kurata reports that an expression map has been constructed using cDNA mapping as a base. This map includes information on tissue-specificity, distribution of isozyme genes, gene families, and functionally related genes in the genome, such as ribosomal protein genes and the histone gene family.

Physical mapping in the Japanese Rice Genome Program will be used to identify economically important genes. Two YAC and three

cosmid clone libraries have been developed, representing about 20% of the rice genome. Ordered libraries will be prepared from these clone libraries. To date, 120 YAC end-clones have been isolated and end-clone mapping is underway. Typical YACs have 400 kb inserts, which, when finished, the Japanese expect will cover the rice genome six times over.

Chromosomes 1, 4, 6, and 11 are being given high priority. It is known that a number of important resistance genes reside on Chromosome 6. Mapping data from the Japanese program have been entered onto two versions of an internal database called RiceBase. One version contains mostly cDNA information, while the other version has the physical map data.

International collaboration of rice mapping efforts was encouraged by an informal workshop held in conjunction with the conference. Dr. Susan McCouch, Cornell University, and Dr. Gou-fan Hong, Director, Chinese Rice Genome Program, co-chaired the session. Highlights included Dr. Kurata's announcement that the Japanese mapping data should be made public later this year. Five prime sequence data for several hundred markers are currently available. Pamela Ronald, University of California, Davis, CA, announced the public availability of a variety of libraries, including those on Bacterial Artificial Chromosomes and cosmids.

Physical Mapping

Physical mapping was again highlighted in the *Arabidopsis* workshop. Caroline Dean (John Innes Institute, Norwich, England) and Howard Goodman (Massachusetts General Hospital) reported that chromosomes 4 and 5 are nearing completion in their joint effort to integrate the two YAC and cosmid maps. A new YAC library developed by David Bouchez should help in developing the integrated physical map. Several thousand *Arabidopsis* cDNAs have been sequenced by the French EST project. Michel Delseny, (CNRS, Perpignan, France) who reported on the project, indicated that the cDNA sequences have been deposited in the public database EMBL.

> *"He defined this era as a new voyage of the Beagle"*

Resources

Plant Genome II provided participants with information on useful technologies and resources. The latest developments in the plant genome databases were outlined, as well as computational tools for mapping and sequence analysis. Database demonstrations with a live Internet link were available throughout the meeting, allowing hands-on experience for interested researchers. Electronic BIOSCI newsgroups were the focus of several workshops organized by Dave Kristofferson (Intelligenetics, Mountain View, CA).

QTL Experimental Design

Quantitative trait loci (QTL) analysis was examined with attention to experimental design and analysis. Dave Webb, Pioneer Hi-Bred, Johnston, IA, looked at soybean cyst-nematode resistance; one soybean introduction was found to have more resistance than any other soybean tested to date. Three resistance loci were identified; with this information, the effect of population size in detecting the traits was tested. Large sample populations were found to be essential in finding and mapping these traits. The minimum sample population size is 200.

The need for large sample populations was again emphasized by Karl Lark, University of Utah, Salt Lake City. Lark found that specialized statistical methods and graphing were needed to identify many important loci. Specifically, Lark identified in interacting traits a condition called epistasis. One trait measured on its own had no effect on plant height. This same trait was found to interact with another plant height QTL and could explain 25% of the plant height variation. The basis of Lark's technique is to use large population sizes and to conduct pairwise comparisons of loci in plants with extreme phenotypes. The results are graphed and epistatic interactions are then identified.

According to Thomas Cheesbrough, South Dakota State University, Brookings, this type of analysis will be essential to studying the genes of such metabolic pathways as oil production, because each enzyme is highly interdependent on the gene products of the entire metabolic chain.

Mapping Technologies

Mapping technologies were featured in several talks and posters throughout the conference. Perry Cregan, USDA, ARS, Beltsville, MD, and

Touching Base with Lisa Lorenzen

Soybase News

Dr. Lisa Lorenzen
Department of Zoology and Genetics
Iowa State University
Ames, IA

The Soybase staff has been concentrating on the metabolic portion of the database. Information (current through summer 1994) on the enzymes and reactions/pathways involved in nitrogen metabolism have been entered and became available August 15, 1994. The complete set also will include references for all cited articles and books.

The information on Fatty Acids is 50 percent collected and entered and is slated for completion early in 1995. A section on nodulins has been added.

The Pathology section continues to grow, with downy mildew, powdery mildew, frogeye leaf spot, potato mosaic virus, cowpea mosaic virus, and stem canker being the latest additions. Soybase staff members have collected and entered information from the literature on both quantitative and qualitative trait linkages to molecular markers. We wish to encourage anyone who has data that he/she wants to include in Soybase to contact us at the E-mail address listed below. We would be happy to include your data.

Thinking toward an integrated legume database, the Soybase staff has assisted in the initiation of four new ACE-type databases: alfalfa, peanuts, common bean, and cool season food legumes such as lentil and chickpea. These databases are being administered under the supervision of Dr. Daniel Skinner, USDA-ARS, Kansas State University; Dr. Gary Kochert, University of Georgia; Dr. Phil McClean, North Dakota State University; and Dr. Fred Muehlbauer, USDA-ARS, Washington State University; respectively.

For additional information on Soybase, or for instructions on how to contact one of the new legume databases, please send inquiries to curator@mendel.agron.iastate.edu.

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Conference--cont. from page 13

others reported on the continued success with simple sequence repeats (SSR). The SSRs are small sequence patterns which are repeated at variable lengths. The variable length of the repeats provides a means to identify varieties and individuals; tools needed by crop breeders and geneticists. In addition to SSR technology, amplified fragment length polymorphism (AFLP), a related new technology, was reported by Drs. Pieter Vos and Marc Zabeau, KeyGene, Wageningen, The Netherlands.

AFLP will provide markers for those map regions which other markers have not successfully bridged. The AFLP technique has the capacity to exploit multiple forms of variation within the genome. The new technology described by Vos is still a long way from direct application by plant breeders, as discussed at the International Triticale Mapping Initiative meeting held in San Diego in conjunction with the Plant Genome II conference.

Plant Genome III

Plant Genome III will be held January 15-19, 1995, in San Diego, CA. Sessions will address all aspects of mapping, from QTLs to the latest molecular marker technologies, instrumentation, and gene isolation. For more information or program suggestions, contact Jerome Miksche or Stephen Heller, USDA/ARS, BARC-W, Bldg. 005, Room 331-C, Beltsville, MD 20705 USA. (See article, "Announcing Plant Genome III" in this issue.)

◆

The Class of 1993 Plant Genome Grant Recipients

G. Agrios, P. Chourey
University of Florida
Molecular and Physiological Genetics of Sucrose Metabolizing Enzymes in Maize Endosperm

M. Alleman
Duquesne University
Regulatory Mutants of the Maize R Locus

J. Anderson, F. Francl
North Dakota State University of Agriculture & Applied Science
Molecular Mapping of Tan Spot Resistance Genes in Wheat

Z. Avramova
Purdue Research Foundation
Nuclear Matrix and Matrix-Attachment Regions (MARs) in Higher Plants

P. Baenziger, Y. Yen
Nebraska Agricultural Experiment Station, University of Nebraska
Exploring the Interface of Qualitative and Quantitative Genetics

W. Baird, A. Abbott, R. Ballard, W. Bridges
Clemson University
Chromosome Mapping in Peach and its Application to Fruit Quality Maintenance

G. Bates
Florida State University
Targeting of DNA Integration to Specific Sites in Plant Chromosomes

J. Birchler
The Curators of the University of Missouri
Molecular Analysis of Maize Centromeres

D. Bisaro
Ohio State University Research Foundation
Molecular Mechanisms of Geminivirus Replication

T. Blake, L. Talbert
Montana State University
Molecular Markers to Correct Germplasm Deficiencies in Wheat and Barley

W. Briggs
Gordon Research Center
1993 Gordon Research Conference on Plant Molecular Biology Signal Transduction and Membrane Proteins

R. Brown, I. Saxena
University of Texas
Molecular Analysis of Cellulose Biosynthesis in Acetobacter xylinum

S. Brown, H. Aldwinckle, N. Weeden
Cornell University
Genome Mapping & Gene Tagging in Apple

M. Bustos
University of Maryland, Baltimore County
Hormonal Control of Gene Expression During Seed Maturation

R. Cantrell
Agricultural Experiment Station
Board of Regents of New Mexico State University
Use of RAPD Markers to Determine the Genetic Diversity of Gossypium Germplasm Derived from Interspecific Hybridization

A. Cheung
Yale University
Molecular and Cellular Analysis of a Pro- and Cys-rich Protein Family in the Pistil

J. Chory
Salk Institute for Biological Studies
Molecular and Genetic Analysis of *Arabidopsis* DET2 Gene

J. Colbert
Iowa State University of Science and Technology
Phytochrome mRNA Degradation: Cis-elements, Factors, and Pathway

S. Colby
Individual Awardee
Regulation of Glandular Trichome-based Insect Resistance in Lycopersicon

D. Cook, K. VandenBosch
Texas A&M Research Foundation
A Peroxidase Gene Induced During Nodule Initiation in Medicago truncatula

M. Cordonnier-Pratt, L. Pratt
University of Georgia Research Foundation, Inc.
Phytochrome and Potential Photomorphogenic Loci in the Grasses

K. Coschigano
New York University
Characterization of Two Fd-GOGAT Genes and Their Roles in Photorespiration

P. Cregan, J. Specht, R. Shoemaker
USDA/ARS Beltsville Area
An Integrated Microsatellite, RFLP, and Conventional Linkage Map of Soybean

H. Daniell
Auburn University
Transformation and Foreign Gene Expression Studies Using the Gene Gun

J. Demski, R. Jarret
University of Georgia Research Foundation, Inc.
Protoplast-Mediated Transformation of Peanut for Virus Resistance

R. Dewey, P. Sisco, D. Danehower
North Carolina State University
The Glossy-15 Gene of Maize, a Cell-Specific Regulator of Leaf Epidermal Traits

J. Doebley
The Regents of the University of Minnesota
Genetics of Inflorescence Development in Maize and Teosinte

X. Dong
Duke University
Molecular Genetic Analysis of Systematic Acquired Resistance in A. thaliana

H. Dooner
DNA Plant Technologies, Inc.
A Set of Maize Lines Carrying Ac at Mapped Locations Dispersed in the Genome

J. Dvorak
The Regents of the University of California
Recombination Between Homoelogous Chromosomes in Wheat

V. Dzelzkalns
Case Western Reserve University
Regulation and Mechanism of the Self-Incompatibility Response of Flowering Plants

E. Earle
Cornell University
Chromosome-Specific Libraries of Tomato

S. Farrand
The Board of Trustees of the University of Illinois
Cis- and Trans-Acting Functions Mediating Ti Plasmid Transfer

R. Ferl
University of Florida
Chromatin Structure and Gene Expression in Plant

D. Gallie
The Regents of the University of California
Isolation of RNA-Binding Proteins Involved in Regulating Translation

S. Gelvin
Purdue Research Foundation
Cell Biology of T-DNA Transfer to Plant Cells

P. Gepts
The Regents of the University of California
Mapping Genetic Determinants of Host-Bacteria Interactions in Common Bean

R. Gilbertson, W. Lucas
The Regents of the University of California
Molecular and Cellular Analysis of Vascular Function Using a Phloem-Limited Virus

B. Gill
Kansas State University
The Sub-Arm Aneuploids of Common Wheat

P. Green
Michigan State University
Control of mRNA Stability in Dicotyledonous Plants

A. Grossman
Carnegie Institution of Washington
The Use of Cyanobacteria to Explore Basic Biological Processes — 1993 Workshop

L. Hadwiger
Washington State University
Genetic Engineering of Non-Host Resistance in Plants

L. Hanley-Bowdoin
North Carolina State University
DNA Replication in Plants: An Accessory Factor for Geminivirus Replication

M. Hanson
Cornell University
Regulation of Synthesis of Plant Mitochondrial Proteins

D. Harry, D. Neale
PSWFRES, USDA, Forest Service
Codominant PCR-based Markers for Pines and Other Conifers

G. Hart
Texas A&M Research Foundation
Construction of an RFLP-Based Genetic Map of Sorghum Recombinant Inbred Lines

T. Hodges, L. Lyznik
Purdue Research Foundation
Gene Targeting of Plant Cells

S. Howell
Boyce Thompson Institute for Plant Research, Inc.
Isolation of Genes Involved in Cytokinin Responses in Arabidopsis

A. Huang
The Regents of the University of California
Molecular and Cell Biology of Oil Bodies in Maize and Brassica

S. Hulbert
Kansas State University
Analysis of the Rp1 and Rp3 Loci of Maize

S. Hulbert, D. Delaney, B. Gill
Kansas State University
Development of a High Density Chromosome Map Using Region-Specific Libraries

A. Hung
The Regents of the University of California
Molecular and Cell Biology of Oil Bodies in Maize and Brusque

R. Innes
Indiana University
Molecular Cloning of Disease Resistance Genes from Arabidopsis and Soybean

A. Jagendorf
Cornell University
Function and Regulation of Chloroplast REC-A Protein

M. James, A. Myers
Iowa State University of Science and Technology
Isolation and Characterization of the Maize Gene Sugary1, a Determinant of Starch Composition in Kernels

J. Kermicle, W. Eggleston
Board of Regents of the University of Wisconsin System
Tests for Ac/Ds-Induced Gene Conversion in Maize

R. Kesseli
University of Massachusetts
Genome Evolution and the Organization of Disease Resistant Genes in the Compositae

J. Kikkert, J. Sanford
Cornell University
Biological Projectiles for Delivery of High Molecular Weight DNA to Plants

A. Kleinhofs
Washington State University
High Resolution Map of the Barley Sub-Telomeric Region Including Rpgl Gene

J. Kohn
The Regents of the University of California, San Diego
QTL Analysis of Developmental Traits in Wild Rice

M. Kuchka
Lehigh University
Nuclear Gene Products and Chloroplast Gene Expression

C. Lamb, C. Ryan
Federation of American Societies for Experimental Biology
FASEB Summer Research Conference: Signal Transduction in Plants

M. Lee, R. Wise
Iowa State University of Science and Technology
Genetic Organization of Resistance to Puccinia coronata in Avena

B. Liu, D. O'Malley, F. Bridgewater, D. Grattapaglia
North Carolina State University
Genome Map Assisted Plant Breeding (GMAPB) for Forest Tree

S. MacKenzie
Purdue Research Foundation
Arabidopsis Mitochondrial Genome Alterations in Response to Nuclear Genotype

P. Maliga
Rutgers, The State University
A Transgenic Approach to Dissect Light Regulation of the Plastid psbD/C Operon

P. Maliga
Gordon Research Center
Gordon Research Conference on Plant Cell & Tissue Culture: Plant Transgenes-Tools for Discovery and Design

D. McCarty, I. Vasil
University of Florida
Viviparous-1 Mediated Repression of Alpha Amylase Genes in Developing Aleurone

S. McCouch
Cornell University
High-Density Genetic Mapping of the Rice Genome Based on Sequence Tagged Microsatellite Sites

M. Mutschler
Cornell University
Genetic Control and Field Efficacy of Acylsugar Mediated Multiple Pest Resistance

J. Nasrallah
Cornell University
A Structural and Transcriptional Analysis of the S-Locus Region of Brassica

D. Neale, N. Wheeler
PSWFRES, USDA, Forest Service
Molecular Marker and Quantitative Trait Mapping in Douglas-Fir

R. Newton
Texas A&M Research Foundation
Conifer Transformation with Shoot Apices and Agrobacterium

H. Nguyen, D. Rosenow
Texas Tech University
Tagging Drought Tolerance Traits in Grain Sorghum Using Molecular Markers

C. Opperman, M. Conkling
North Carolina State University
Characterization of a Nematode-Responsive Plant Gene Promoter

P. Ozias-Akins, W. Hanna
University of Georgia Research Foundation, Inc.
Development, Genomic Diversity, and Gene Expression in Aposporous Genotypes

G. Phillips, G. Kuehn, S. Bagga
Agricultural Experiment Station
Board of Regents of New Mexico State University
Isolation of Genes Coding for Plant Polyamine Biosynthetic Enzymes

G. Powell, A. Abbott
Clemson University
Characterization of the 12-Desaturase

L. Pratt, M. Cordonnier-Pratt
University of Georgia Research Foundation, Inc.
Phytochrome Gene Family in Tomato

C. Qualset
The Regents of the University of California
Research Collaboration Group on Molecular Mapping in Wheat and Its Relatives

R. Rajasekharan, J. Kemp
Agricultural Experiment Station
Board of Regents of New Mexico State University
Lysophosphatidic Acid Acyltransferase: Enzyme and Gene Isolation from Soybean

L. Ream
Oregon State University
A Multifunctional DNA Binding Protein Required for Gene Transfer to Plants

K. Redman, M. Johnson
University of Alabama
A Novel Mechanism for Ribosomal Protein Modulation: On/Off Splicing

P. Ronald
The Regents of the University of California
Map-based Cloning in Rice

L. Rowland
USDA/ARS Beltsville Area

Tagging Genes which Control Chilling Requirement in a Woody Perennial

M. Saghai Maroof
Virginia Polytechnic Institute and State University
Assessment of Barley Germplasm Using Nuclear and Organellar Molecular Markers

R. Schmidt, M. Yanofsky
The Regents of the University of California, San Diego
An Analysis of Floral Regulatory Genes in Maize

S. Scofield
The Regents of the University of California
Promoters to Express Ac Transposase for Efficient Tagging Systems

E. Signer
Massachusetts Institute of Technology
Repeat-Induced Gene Silencing in Arabidopsis

S. Strauss, W. Rottmann
Oregon State University
Floral Homeotic Genes for Genetic Engineering of Sterility in Populus

C. Stuber
USDA/ARS South Atlantic Area
Stability of QTL Mapping in Maize Under Varying Environmental Stresses

T. Sullivan
Board of Regents of the University of Wisconsin System
Molecular and Biochemical Analysis of the Maize Brittle-1 Gene

S. Sun
University of Hawaii, Manoa
Genetic Transformation of Grain Legumes for Improved Protein Quality

R. Thornburg
Iowa State University of Science and Technology
Selection for Second Site Mutations in the Wound-Induction Pathway

J. Trumble
The Regents of the University of California
Transgenic Insect-Resistant Brassica with Glossy Wax Genes from Arabidopsis

R. Vierstra
Board of Regents of the University of Wisconsin System
Molecular and Biochemical Analysis of Ubiquitin Conjugating Enzymes in Higher Plants

C. Weil
University of Idaho
Transposable Element-Mediated Dissection of Protein Structure and Function

R. Wing, A. Paterson
Texas A&M Research Foundation
Physical Mapping and Map-Based Cloning in Polyploids: Cotton as a Model System

R. Wise
USDA/ARS Mid-West Area
High Resolution Mapping of the M1-a Disease Resistance Locus in Barley

S. Yang
The Regents of the University of California
ACC Malonyltransferase: Isolation, Characterization and Molecular Cloning

J. Yoder
The Regents of the University of California
Transposon Mutagenesis in Tomato

R. Zielinski
The Board of Trustees of the University of Illinois
Molecular Characterization of CaBP-22, a Leaf-Specific, EF-Hand Ca2+ -Binding Protein

IMPORTANT

1995 NRI Grant Deadlines Announced

See page 8 of this issue

Touching Base with Bradley Sherman

A Primer on Images and the Internet

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High-speed digital networks and computers with graphical capabilities have made it possible to retrieve and view images almost as easily as one views purely textual material. This article is intended to be a quick introduction to this subject with some pointers to other resources.

What Is a Digital Image?

Digitally stored images are analogous to a paint-by-number kit. The image is composed of rectangular regions called pixels, and each pixel is assigned a number. Each number corresponds to a color or shade of grey. In order to reconstruct an image for viewing, one needs both the values for each pixel and a legend which maps the numerical values to a color. Image files on computers typically contain both the array of pixel values and the color map.

Digital images usually contain less information than a corresponding image captured with conventional photographic methods. Photographic film is an excellent repository for information. One 35mm slide can easily hold one 100 million bytes of data. This makes the digital storage of images somewhat problematic. Even if the cost of storage were not a factor, the time involved in retrieving and displaying very large stored images would be prohibitive. All common conversion of photographic data to digital data involves the loss of some information. Once the images are digitized, they are often compressed. Some compression methods result in further information loss. There is always a tradeoff between faithfulness of reproduction and amount of storage space required. Even if storage were free, it would be advantageous to keep the image size small for rapid transferal and viewing. This dynamic has led to many different formats for computer images, each with advantages and disadvantages. The decision about which format to use is highly dependent on the application, and on the hardware that will be used. On the Internet, exchanged images tend to follow two main formats: GIF and TIFF. These formats, particularly the latter, have many variations. JPEG images are a ubiquitous TIFF variant (JFIF).

> *"What is the use of a book," thought Alice,*
>
> *"without pictures or conversations?" — Lewis Carroll*

In addition to these, different computer types can have their own internal formats, customized for their particular hardware. The PICT format on Macintosh is an example of this.

Software

There is software available for Unix, Macintosh and Intel-based platforms that will allow you to view downloaded images. You may retrieve them using anonymous ftp and experiment with them for free. Some of the software is shareware, and the author expects some small compensation if you like the software and continue to use it. Sources for image

viewing software for major platforms are listed at end of this article.

Hardware

Workstations such as those from Sun Microsystems or Silicon Graphics were developed with advanced graphical uses in mind. High-end Macintoshes and PC-clones are adequate, however. Monitor screens should be at least 15 inches, and graphics hardware may be helpful or necessary. Image analysis can be slow even on very fast computers.

Typical Image Sizes and Network Bandwidth

Images seen on the Internet can range in size from hundreds to millions of bytes. A four-megabyte file is not uncommon. Internet connections can be characterized by the bandwidth of the connection. Typical bandwidths are 9.6, 14.4, and 56 kilobaud. Major nodes may have one megabaud connections or better. Bytes per second can be approximated by dividing the baud rate by 10. A 56 kilobaud channel will transfer about 5,600 bytes per second.

A typical 100 kilobyte image will take at least 1 second to move across a 1 megabaud channel and more than 17 seconds across 1 of 56 kilobaud. In addition, the presentation of the image on the computer screen may take seconds once the image has been transferred.

To the user, large images or slow network connections will be seen as delays. An occasional 10-second delay after pressing a key or mouse can be tedious in an interactive environment, arguing for very high speed network connections or using smaller image files. Compression techniques can be very useful, particularly when animated images are transferred.

Image Capture

There are at least two sorts of devices which one can use to digitize an image. Both make use of charge-coupled devices (CCD) which use quantum effects to transduce light to a pattern of electrical signals. Scanners have a linear array of CCDs which are mechanically moved across a flat image (in the manner of a xerographic machine). CCD cameras have a two-dimensional array of devices so that the entire image is captured at once. CCD cameras use conventional optics and hence can be used to take pictures of 3-D objects.

CCD devices are susceptible to thermal noise; they will produce small random signals even in absolute darkness. To increase signal-to-noise ratios, the devices can be cooled. Some CCD cameras come with refrigeration units for this purpose. CCD cameras are more expensive, but allow for a higher throughput in a production setting.

Commercial photographic processing labs commonly have equipment to directly digitize color slides. Copy stores will often have scanners available for rental. These work quite well with color or black-and-white prints.

Where To Get Image Viewing Software

All of this software may be retrieved using anonymous ftp.

Macintosh
JPEGView
ftp to sumex.aim.stanford.edu
directory: /info-mac/app/

NIH Image
ftp to zippy.nimh.nih.gov
directory: /pub/nih-image/

Unix/X11
xv
ftp to bongo.cc.utexas.edu
directory: /gifstuff/xwindows/
xloadimage
ftp to bongo.cc.utexas.edu
directory: /gifstuff/xwindows/

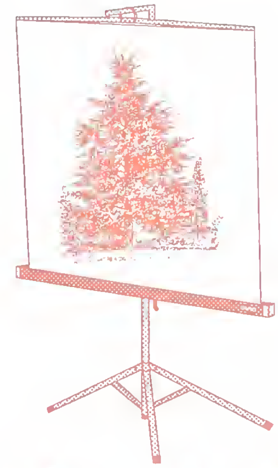
Intel/Windows3
Lview
ftp to oak.oakland.edu
directory: /pub/msdow/windows3/

For More Information

These Usenet electronic conferences are sources of useful discussion:

alt.binaries.pictures.misc
comp.graphics
alt.graphics.pixutils

Lists of Frequently Asked Questions (FAQ), with answers, for these conferences are available by anonymous ftp from rtfm.mit.edu. ◆



Maize Genome Database, a USDA-ARS Plant Genome Database

Mary Polacco, Database Developer
Curtis Hall, University of Missouri
Columbia, MO 65211

The Maize Genome Database, or MaizeDB, is curated as a Sybase database at the University of Missouri-Columbia and provides user-friendly, Internet access to the maize genome and the biology of maize.

Information includes 142 genetic maps, with 4,864 mapped loci, recombination and map score data (2,164 entries), 986 probes, 1,701 genetic/cytogenetic stocks, 7994 locus variations, 4,662 stock pedigrees, 5,600 bibliographic references indexed to genetic objects, and addresses of maize researchers.

Gene functionality may be queried by mutant phenotype, trait, confirmed or putative gene products, metabolic pathways, induction conditions and text descriptions of genes. Work is in progress to document quantitative trait loci. Users with Internet connections may access the data by several procedures that are described more fully below: guest login, gopher, World Wide Web, or file transfer of a special graphics, ACEDB format.

Data is input from various sources, including specially formatted, electronic lab notebooks of researchers who focus on mapping or mutant characterization. The contributors are international and from academic, government, and industrial research groups. Information, especially regarding gene function and expression, is also taken from the scientific literature, both electronic and printed.

Connecting To Other Databases

Using Mosaic, a type of free software that connects users to information on the World Wide Web (WWW), users connected to MaizeDB may retrieve information, including images and graphics, from other databases around the world, as easily as if the data existed in the MaizeDB records. If desired, they may store the information on their machines.

For example, while browsing the MaizeDB, you may read that the function of maize gene, dps1, was confirmed by transgenic complementation of E. coli mutations in dap1. By clicking on dap1, you would retrieve the record from the E. coli Stock Center at Yale University. Clicking on the EC number for gene products that are enzymes connects you to the ENZYME database. ENZYME describes the reaction, and in turn, connects to all Swiss-Prot entries corresponding to that EC number, as well as to OMIM (On-line Mendelian Inheritance in Man).

WWW connectivity requires precise matching of records in MaizeDB to records in other databases around the world. It permits curators of distinct datasets to combine data in a seamless fashion without actually importing the data. The ability to extract distinct formats of data from MaizeDB makes preparing files of matching identifiers relatively easy, so that external databases may use the WWW to connect to us, as occurred in June 1994 with SwissProt.

WWW/MaizeDB currently accesses external data in the following databases:

GenBank.......nucleotide sequences
SwissProt.....protein sequences; connects to Prosite (motifs, signature sequences), MedLine, EMBL
dbEST.........random cDNAs partial sequences, with periodically updated homology searches
E. coli Stock Center.......E. coli genetic stocks, map
ENZYME.......reactions, comments; connects to SwissProt, OMIM
AAtDB........*Arabidopsis* Genome Database

Accessing MaizeDB-Requirements

Guest login only requires that your machine have Internet connectivity, direct or indirect. Modem connections are supported, as are connections using any computer, including

PC, Macintosh, and Unix.
guest login protocol:
telnet teosinte.agron.missouri.edu
login: guest
password: corncob
Guest login provides access to:

- gopher
- MaizeDB, a Sybase database; access provided to users with either X-Window or vt100 emulation
- Lynx, a WWW browser that does not require an X-Window; it does not support mouse-capability
- help

Guests are encouraged to leave comments on the Note form of the database. While not required, leaving your e-mail address will permit us to contact you directly for further clarification.

NOTE: Users with X-Windows (this is not the same as Microsoft Windows) software will enjoy the most user-friendly access to the database. If connecting by modem, the X-Window will not function, and users should select the vt100 emulation.

NOTE: If using the vt100 emulation of MaizeDB/Sybase, type "r" while holding down the "control" (aka "CTRL") key to access the commands required to query or browse the database. The command utilities are described in more detail in the "help" option that appears or after successful login as a guest.

Gopher

Gopher makes available hierarchical collections of information across the Internet. Gopher client (user) software provides easy access to all gopher data servers. All words in a record, except commonly used words, are indexed and thus may be used to query records.

Free gopher client software for Unix, PC, or Macintosh machines is available by anonymous ftp (file transfer protocol)* from boombox.umn.edu. Once installed, open server teosinte.agron.missouri.edu, port 70 or use gopher to find us by location in Columbia, Missouri. On-line help is provided by the gopher software and is in a file on the MaizeDB gopher server.

World Wide Web (WWW)

WWW is a hypermedia retrieval system which allows users to traverse on-line documents by clicking on hyperlinks-terms, icons, or images that point to other related documents. Hyperlinks permit retrieval of any document anywhere on the Internet. Retrieved "documents" may include text files, graphics, and videos. Connecting to the WWW currently works best if users have access to Mosaic software installed on a Unix machine. Macintosh and PC Mosaic software are rapidly approaching the capability of the Unix version.

Users without WWW software may access the WWW-linked format of MaizeDB by selecting the Lynx option after "guest login."

Mosaic software supports mouse capability and is available without charge by anonymous ftp from ftp.ncsa.uiuc.edu. The Unix version, but not the Macintosh or PC version, requires an X-Window on the user's machine; it will require a systems administrator to install. To access MaizeDB from Mosaic software, use our WWW address, otherwise known as URL or uniform resource locator:
http://teosinte.agron.missouri.edu/top.html

The WWW formatted data is dynamically extracted from the most current version of the database, which is continuously updated.

ACEDB Format

This is a special graphical format and requires a UNIX machine. The database may be retrieved by anonymous ftp from the National Agricultural Library, probe.nalusda.gov in directory pub/maize. This format is static, and periodically extracted from MaizeDB. It does not support the robust queries of the Sybase database, accessible by the guest login service.

ANONYMOUS FTP requires that the user have ftp or file transfer software to connect to another machine. Once connected, login as "anonymous" and use your e-mail address as the password. If using a Unix machine, type: cd pub/maize, and to transfer the database, type: get mace.tar.Z

History Of MaizeDB Design: Some Landmarks

Fall 1991

First prototype MaizeDB operational. Some 24,000 records created the first 6 months, largely from data summaries in the Maize Genetics Cooperation Newsletter (MNL), volume 65. The database currently contains over 78,000 records.

December 1992

First public access to the data, a gopher server established. First access was 100-200 connections/month, and has grown to over 1,000 connections/month.

March 1993

Maize Gene List, MNL, vol 67, pp. 134-15, extracted from MaizeDB Version 2 of MaizeDB implemented.

June 1993
ACEDB formatted data extracted from MaizeDB.

August 1993
Tool developed for loading references from PC and Macintosh reference manager formats.

December 31, 1993
Guest login to MaizeDB established.

Winter 1994
MaizeDB placed on the World Wide Web; currently there are 600-1,100 connections/week.
WWW connections made to external databases, listed above.

March 1994
Genetic indexing of 1993 references extracted from the MaizeDB, published as hardcopy in MNL, vol 69. pp 148-153. Information was indexed to chromosome, gene or allele and trait.

June 1994
SwissProt connects to MaizeDB using a file extracted from MaizeDB per specifications of SwissProt curators.

Developers and Curators of the Database Include:

E.H. Coe (PI), P. Byrne, G. Davis, D. Hancock, M. Polacco (Columbia, MO)
M. Berlyn, S. Letovsky (New Haven, CT)
C. Fauron (Salt Lake City, UT)
S. Rodermel, C. Wetzel (Ames, IA)
M. Sachs (Urbana, IL)
For further help in accessing the database, please e-mail
db_request@teosinte.agron.missouri.edu
or contact Denis Hancock,
(314) 882-1722 (phone)
(314) 874-4063 (FAX) ◆

Introducing Dr. Edward Kaleikau

Dr. Edward K. Kaleikau has assumed responsibility as program director for the Plant Genome Program of the USDA National Research Initiative Competitive Grants Program (NRICGP). In this position, Dr. Kaleikau coordinates the competitive grant review process for the Plant Genome Program. His responsibilities include selecting and working with members of the review panel in conjunction with the panel manager, as well as handling other review assignments as needed.

In addition, Dr. Kaleikau is co-chairman of the Plant Genome Steering Committee along with Dr. Jerome Miksche, and serves on the USDA Biotechnology Research Subcommittee.

Dr. Kaleikau, a native of Hawaii, received his B.S. degree in biology/chemistry (1981) from Graceland College in Lamoni, IA and Ph.D. degree in plant genetics (1988) from Kansas State University. His Ph.D. dissertation investigated the inheritance and chromosomal mapping of genes controlling in vitro tissue culture response in wheat. Dr. Kaleikau developed his interest in plant genetics during an internship at the Arco Plant Research Institute in Dublin, CA, after graduation from college. He gathered further experience as a technician for Advanced Genetic Sciences in Manhattan, KS.

Prior to joining the NRICGP, Dr. Kaleikau received postdoctoral training at Stanford University, where he was awarded fellowships from both the National Institute of Health and National Science Foundation to study the regulation of transcription initiation and termination of rice mitochondrial genes.

Dr. Kaleikau can be contacted as follows:

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Phone: (202) 401-5114
USDA/CSRS/NRICGP
901 D Street, SW
Aerospace Bldg, Rm 323
Washington, DC 20250-2241 ◆

Announcing Plant Genome III Meeting

Building on the successes of Plant Genome I and II, we are pleased to announce that the Plant Genome III meeting will be held on January 15-19, 1995, in San Diego, CA.

Session Topics:

1. Comparative Genetic Mapping
2. Isolation and Transformation of Agriculturally Important Genes
3. Instrumentation/Technology
4. Applications of cDNA Research
5. Chromosome Structure
6. ALFPs/QTLs/Metabolic Pathways

In addition to the formal sessions and posters during the week, Sunday afternoon will feature a computer workshop on Genome Information Tools and Resources. The workshop will provide a view of some of the existing software used to maintain and analyze genomic information. The talks at this workshop are designed to give the average plant molecular biologist an idea of available resources and their capabilities. "Hands on" computer sessions will also be available throughout the week. Don't miss this opportunity for individualized training.

Additional Workshops:

Sunday, January 15: 9:00 am - 6:00 pm

International Consortium for Sugar Cane Biotechnology
Organized by James Irvine (JIRVINE@TAMU.EDU)

Tuesday, January 17: 3:30 pm - 6:00 pm

1. Pine Tree - Part I
   Organized by Dave Neale
   (DBN@S27W007.PSWFS.GOV)
2. Rice
   Organized by Susan McCouch
   (SUSAN_MCCOUCH@QMRELAY.MAIL.CORNELL.EDU)
3. BIOSCI
   Organized by Dave Kristofferson
   (KRISTOFF@NET.BIO.NET)
4. *Arabidopsis*
   Organized by Caroline Dean
   (ARABIDOPSIS@BBSRC.AC.UK)
   Howard Goodman
   (GOODMAN@FRODO.MGH.HARVARD.EDU)
5. Barley
   Organized by Patrick Hayes
   (HAYESP@CSS.ORST.EDU)

Tuesday, January 17: 7:30 pm - 10:00 pm

1. Pine Tree - Part II
   Organized by Dave Neale
   (DBN@S27W007.PSWFS.GOV)
2. Grass Genome Integration
   Organized by Jeff Bennetzen
   (MAIZE@BILBO.BIO.PURDUE.EDU)
   Michael Gale (JEFFERY@BBSRC.AC.UK)
3. Nomenclature
   Organized by Carl Price
   (PRICE@MBCL.RUTGERS.EDU)
   Ellen Reardon
   (REARDON@CCIT.ARIZONA.EDU)
4. Tree Fruit
   Organized by Sriyani Rajapakse
   (SRIYANI_RAJAPAKSE@QUICKMAIL.CLEMSON.EDU)
5. BIOSCI (Repeat of afternoon session)
   Organized by Dave Kristofferson
   (KRISTOFF@NET.BIO.NET)

Wednesday, January 18: 3:30 pm - 6:00 pm

1. Maize
   Organized by Ed Coe
   (ED@TEOSINTE.AGRON.MISSOURI.EDU)

2. Legumes
Organized by Randy Shoemaker
(RCSSHOE@IASTATE.EDU)
3. ITMI
Organized by Calvin Qualset
(ITMI@UCDAVIS.EDU)
Olin Anderson (OANDERSON@PW.USDA.GOV)
Pat McGuire (ITMI@UCDAVIS.EDU)
Michael Gale (JEFFERY@BBSRC.AC.UK)
4. Tagging Genes for Abiotic Stress
Organized by Henry Nguyen (806-742-1622)
5. Cotton
Organized by Andrew Paterson
(AHP2343@BIOCH.TAMU.EDU)

Abstract Deadline:

Abstracts submissions are due by November 1, 1994. All submitted or invited poster talks will be one-page long, using forms provided by the PG-III conference organizer, Scherago International. The PG-III abstracts will be available online prior (and after) to the meeting at probe.nalusda.gov via gopher and the WWW.

Student Travel Grants:

The International Society for Plant Molecular Biology is again sponsoring four student travel grant awards. For details please contact Dr. Stephen Heller by E-mail.

Location: Town & Country Hotel
500 Hotel Circle North
San Diego, CA 92108
Phone: (619) 291-7131
FAX: (619) 291-3584

Cost: $300 advance registration up to December 1, 1994
$350 after December 1, 1994 and on-site
$100 Student (Pre-Ph.D) registration
(Requires a letter of certification from department chairperson)

All registrations include one copy of the printed conference abstracts, Monday-Thursday continental breakfasts, Sunday evening opening reception, Monday evening wine & cheese reception, and Wednesday evening dinner.

PG-III Co-Chairpersons:

Stephen Heller, USDA/ARS, Beltsville, MD, USA
(SRHELLER@ASRR.ARS.USDA.GOV)
Jerome Miksche, USDA/ARS, Beltsville, MD, USA
(JMIKSCHE@ASRR.ARSUSDA.GOV)
Michael Gale, John Innes Centre, Norwich, UK
(JEFFERY@BBSRC.AC.UK)
Susan McCouch, IRRI, Philippines
(SUSAN_MCCOUCH@QMRELAY.MAIL.CORNELL.EDU)

Conference Co-sponsors:

USDA, Agricultural Research Service
USDA, National Agricultural Library
Rockefeller Foundation
International Society for Plant Molecular Biology
John Innes Centre

To Register Contact:
Darrin Scherago
Scherago International, Inc.
11 Penn Plaza, Suite 1003
New York, NY 10001
Phone: (212) 643-1750
FAX: (212) 643-1758
E-mail: SCHERAGO@BIOTECHNET.COM ◆

Calendar of Upcoming Genome Events

Meetings

September 18-22, 1994: **16th International Congress on Biochemistry and Molecular Biology**, New Delhi, India. Contact: K. Kooraram, Magnet World Travel, 18-30 Clerkenwell Rd., London, EC1M 5NN, UK.

September 19-20, 1994: **Drug Discovery & Commercial Opportunities in Medicinal Plants**, Arlington, VA. Contact: IBC USA Conferences Inc., 225 Turnpike Rd., Southborough, MA 01772-1749. PHN: (508) 481-6400, FAX: (508) 481-7911.

September 19-22, 1994: **John Innes Symposium: Biochemistry of Development**, Norwich, England. Contact: John Innes Centre, Norwich Research Park, Colney, Norwich, Norfolk, England NR4 7UH. PHN: 44 603 52571, FAX: 44 603 56844.

September 25-27, 1994: **Harnessing Apomixis: A New Frontier in Plant Science**, College Station, TX. Contact: Dr. David M. Stelly, Dept. of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474. PHN: (409) 845-2745, FAX: (409) 862-4733, EMAIL: monosom@rigel.tamu.edu.

September 30-October 5, 1994: **Structural Molecular Biology Conference**, Mont St. Odile, France. Contact: Dr. Josip Hendekovic, European Science Foundation, 1 Quai Lezay-Marnesia, F-67080 Strasbourge, Cedex, France. PHN: (88) 76 71 35, FAX: (88) 36 69 87, TELEX: 890440.

October 2-6, 1994: **22nd Aharon Katzir-Katchalsky Conference: Plant Molecular Biology--Potential Impact on Agriculture and the Environment**, Koln, Germany. Contact: Secretariat 22nd AKK Conference, Aharon Katzir-Katchalsky Center, Weizmann Institute of Science, Rehovot 76100, Israel. PHN: 972-8-342148, FAX: 972-8-474425.

October 2-6, 1994: **1994 Second International Symposium on the Applications of Biotechnology to Tree Culture, Protection, and Utilization**, Minneapolis, MN. Contact: Edith Franson, Executive Secretary, Tree Biotechnology Symposium, Forestry Sciences Laboratory, P.O. Box 898, Rhinelander, WI 54501. PHN: (715) 362-7474, FAX: (715) 362-7816.

October 7-10, 1994: **Genetic & Biochemical Approaches for Studying Cell Death, American Society for Biochemistry and Molecular Biology Fall Symposia 1**, Granlibakken, Lake Tahoe, CA. Contact: ASBMB Fall Symposia Office, Room 3206, 9650 Rockville Pike, Bethesda, MD 20814-3998. PHN: (301) 530-7010, FAX: (301) 530-7014.

October 14-17, 1994: **Mechanisms of Regulated Intracellular Protein Degradation: American Society for Biochemistry and Molecular Biology Fall Symposia 2**, Whistler, British Columbia, Canada. Contact: ASBMB Fall Symposia Office, Room 3206, 9650 Rockville Pike, Bethesda, MD 20814-3998. PHN: (301) 530-7010, FAX: (301) 530-7014.

October 16-21, 1994: **Recombinant DNA Biotechnology III Conference**, Deauville, France. Contact: Engineering Foundation, Room 303, 245 East 47th St., New York, NY 10017. PHN: (212) 705-7837, FAX: (212) 705-7441.

October 28-31, 1994: **Oligonucleotide Selection and Molecular Diversity: American Society for Biochemistry and Molecular Biology Fall Symposia 3**, Granlibakken, Lake Tahoe, CA. Contact: ASBMB Fall Symposia Office, Room 3206, 9650 Rockville Pike, Bethesda, MD 20814-3998. PHN: (301) 530-7010, FAX: (301) 530-7014.

November 1-4, 1994: **Cucurbitaceae 94: Evaluation and Enhancement of Cucurbit Germplasm**, South Padre Island, TX. Contact: Dr. James R. Dunlap, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco TX 78596. PHN: (210) 968-5585, FAX: (210) 968-0641, EMAIL: j-dunlap@tamu.edu

November 13-16, 1994: **Third International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Organisms**, Monterey, CA. Contact: Ms. Pat Day, University of California, DANR, 300 Lakeside Dr., 6th Flr., Oakland, CA 94612-3560 OR USDA, Office of Agricultural Biotechnology. PHN: (703) 235-4419, FAX: (703) 235-4429.

November 17-19, 1994: **1994 San Diego Conference: The Genetic Revolution**, San Diego, CA. Contact: Scherago International, 11 Penn Plaza, Suite 1003, New York, NY 10001. PHN: (212) 643-1750, FAX: (212) 643-1758, EMAIL: Scherago@Biotech.Net.Com.

November 21-24, 1994: **Brighton Crop Protection Conference: Pest and Diseases**, Brighton, UK. Contact: Conference Associates and Services Ltd., 55 New Cavendish St., London W1M 7RE, UK.

Workshops and Courses

September 26-30 or October 31-November 4, 1994: **Polymerase Chain Reaction Methodology Workshop**, Columbia, MD. Contact: Exon-Intron, Suite 130, 9151 Rumsey Rd., Columbia, MD 21045-1929. PHN: (301) 730-3984, FAX: (301) 730-3983.

October 3-7, 1994: **RNA Isolation & Characterization Workshop**, Columbia, MD. Contact: Exon-Intron, Suite 130, 9151 Rumsey Rd., Columbia, MD 21045-1929. PHN: (301) 730-3984, FAX: (301) 730-3983.

October 10-14, 1994: **Advanced Course on Molecular Biology Workshop**, Leiden, Netherlands. Contact: Dr. L.A. van der Meer-Lerk, Institute of Biotechnology Studies Delft, Kluyver Laboratory, Julianalaan 67, 2628 BC, Delft, Netherlands. PHN: 015-78 51 40, FAX: 015-78 23 55.

October 17-20, 1994: **Polymerase Chain Reaction Techniques and DNA Sequencing Lecture Course**, Lake Tahoe, NV. Contact: Director, Center for Advanced Training in Cell and Molecular Biology, Catholic University of America, 620 Michigan Ave., NE, Washington, DC 20064. PHN: (202) 319-6161, FAX: (202) 319-4467, EMAIL: millerm@cua.edu.

October 17-20, 1994: **Recombinant DNA Methodology and DNA Sequencing Lecture Course**, Lake Tahoe, NV. Contact: Director, Center for Advanced Training in Cell and Molecular Biology, Catholic University of America, 620 Michigan Ave., NE, Washington, DC 20064. PHN: (202) 319-6161, FAX: (202) 319-4467, EMAIL: millerm@cua.edu.

December 14-17, 1994: **International Symposium on Plant Molecular Biology and Biotechnology Workshop**, New Delhi, India. Contact: G. Chatterjee, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India. PHN: (011) 6867356, FAX: (011) 6862316.

January 7-13, 1995: **Plant Cell Biology: Mechanisms, Molecular Machinery, Signals, and Pathways: Keystone Symposium**, Taos, NM. Contact: Keystone Symposia, Drawer 1630, Silverthorne, CO 80498. PHN: (303) 262-1230, FAX: (303) 262-1525.

Future Events

January 15-19, 1995: **Plant Genome III**, San Diego, CA. Contact: Plant Genome III, c/o Scherago International Inc., 11 Penn Plaza, New York, NY 10001. PHN: (212) 643-1750, FAX: (212) 643-1758, EMAIL: scherago@biotechnet.com

February 4-9, 1995: **Advances in Gene Technology: Protein Engineering and Structural Biology: Miami Bio/Technology Winter Symposium**, Ft. Lauderdale, FL. Contact: Miami Bio/Technology Winter Symposia, P.O. Box 016129 (M823), Miami, FL 33101. PHN: (800) 642-4363, FAX: (305) 324-5665, EMAIL: mbws@mednet.med.miami.edu

March 5-9, 1995: **XVIII Eucarpia Symposium: Ornamental Plant Improvement, Classical and Molecular Approaches**, Tel Aviv, Israel. Contact: Dan Knassim Ltd., P.O. Box 57005, Tel Aviv, 61570 Israel. PHN: (972) 3-5626470, FAX: (972) 3-5612303.

April 23-27, 1995: **3rd International Union of Biochemistry and Molecular Biology Conference: Molecular Recognition**, Singapore. Contact: 3rd IUBMB Conference Coordinator, Ken-Air Destination Management Company, 35 Selegie Rd., 09-19 Parklane Shopping Mall, Singapore 0718. PHN: (65) 336-8857/8, FAX: (65) 336-3613.

May 13-17, 1995: **Ninth International Biotechnology Meeting & Exhibition**, San Francisco, CA. Contact: Biotechnology Industry Organization, 1625 K St., NW,

Suite 1100, Washington, DC 20006-1604. PHN: (202) 857-0244, FAX: (202) 331-8132 or (202) 857-0237.

July 4-7, 1995: **9th International Rapeseed Congress,** Cambridge, England. Contact: Denis Kimber, 44 Church St., Haslingfield, Cambridge, CB3 7JE, England.

July 14-19, 1995: **15th International Conference on Plant Growth Substances,** Minneapolis, MN. Contact: Gary Gardner, Dept. of Horticultural Science, University of Minnesota, 305 Alderman Hall, St. Paul, MN 55108. FAX: (612) 624-3606, EMAIL: ggardner@maroon.tc.umn.edu

August 6-11, 1995: **10th International Workshop on Plant Membrane Biology,** Regensburg, Germany. Contact: Widmar Tanner, Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, Universitätsstrasse 31, 93053 Regensburg, Germany. FAX: 49-943-3352.

August 6-12, 1995: **20th World Congress of the International Union of Forestry Research Organisations,** Tampere, Finland. Contact: Professor Risto Seppala, Finnish Forest Research Institute, IUFRO-95, Secretariat Unioninkatu 40A 00170, Helsinki, Finland.

Speakers and posters will cover various genetic, molecular, physiological, cytological and evolutionary aspects of asexual reproduction through seed and its application to crop improvement.

Harnessing Apomixis: A New Frontier in Plant Science

September 25-27

Hilton Hotel and Conference Center College Station

Contact:

Dr. David M. Stelly
Department of Soil and Crop Sciences
Texas A&M University
College Station, Texas 77843-2474
E-mail: monosom@rigel.tamu.edu
Phone: (409) 845-2745
Fax: (409) 862-4733

Related topics in plant sexual reproduction also will be presented. Some financial support for international attendees will be available.

Survey of Synonymous Codon Usage in Nuclear Genes of *Arabidopsis*, Soybean and Maize

Julia Bailey-Serres and Sheila L. Fennoy
Department of Botany and Plant Sciences, University of California
Riverside, CA 92521-0124

The overall bias in synonymous codon usage of a genome is species-specific. Analysis of protein coding regions of small samples of plant genes for a number of species revealed codon usage biases.[1] The synonymous codon usage of nuclear genes of plants varies mainly in the bias toward C or G *versus* A or U in the silent third nucleotide position. Nuclear gene coding regions of monocots are enriched in codons ending in C and G, whereas dicots have a higher frequency of codons ending in A and U.

We used a multivariate statistical analysis to examine codon usage in maize. More biased codon usage was recognized among more highly expressed genes, whereas more random codon usage was observed among more lowly expressed genes. Our work indicates that the overall codon usage patterns in maize reflect the G+C content of the genome. Codon usage bias of individual genes may not solely reflect the nucleotide compositional bias of a chromosomal region, but may be affected by selection on the silent third nucleotide.[2]

The accumulation of DNA sequence data for a large number of nuclear genes of plants provided an opportunity to further examine synonymous codon usage. Table 1 shows a summary of codon usage for three plant species, maize (*Zea mays* L.), soybean (*Glycine max* L.), and Arabidopsis (*Arabidopsis thaliana*). Non-duplicate protein coding sequences were obtained from the September 1992 releases of GenBank and EMBL databases and the literature, and the relative synonymous codon usage was determined. The synonymous codons used at a higher frequency in these data sets are indicated with an asterisk.

Information on codon usage is useful for the design of degenerate oligonucleotide primers for PCR amplification of regions encoding conserved proteins. In addition, consideration of G+C content or codon usage appears to be important for high levels of expression of bacterial genes in plants.[3,4] Further systematic analyses are needed to determine the role of the G+C content and codon usage in regulating gene expression.

References

1. Campbell, W.H. and Gowri, G. (1990) *Plant Physiol.*, **92**, 1-11.
2. Fennoy, S.L. and Bailey-Serres, J. (1993) *Nucl. Acids Res.*, **21**, 5294-5300.
3. Perlak, F.J., Fuchs, R.L., Dean, D.A., McPherson, S.L. and Fischhoff, D.A. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 3324-3328.
4. Koziel, M.G., Beland, G.L., Bowman, C., Carozzi, N.B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M.R., Merlin, E., Rhodes, R.,Warren, G.W., Wright, M. and Evola, S.V. (1993) *Bio/Technology*,**11**, 194-200.

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Table 1

		Maize		Soybean		*Arabidopsis*	
AA	CODON	N	RSCU	N	RSCU	N	RSCU
Ala	GCU	841	0.92	606	1.5*	1572	1.87*
	GCC	1319	1.45*	376	0.93	653	0.78
	GCA	511	0.56	492	1.22	728	0.87
	GCG	955	1.05	142	0.35	403	0.48
Leu	UUA	102	0.19	215	0.65	338	0.62
	UUG	407	0.75	474	1.44	860	1.36
	CUU	527	0.97	531	1.62*	1024	1.62*
	CUC	993	1.83*	362	1.1	794	1.26
	CUA	220	0.41	161	0.49	359	0.57
	CUG	1000	1.85*	229	0.7	359	0.57
Gly	GGU	640	0.83	508	1.29*	1287	1.5*
	GGC	1417	1.83*	293	0.75	458	0.53
	GGA	476	0.61	498	1.27*	1287	1.5*
	GGG	566	0.73	274	0.7	396	0.46
Val	GUU	512	0.77	634	1.56*	1172	1.58*
	GUC	883	1.33	243	0.6	712	0.96
	GUA	199	0.30	182	0.45	269	0.36
	GUG	1062	1.6*	568	1.4	805	1.09
Ser	AGU	182	0.43	420	1.38*	487	0.93
	AGC	640	1.54*	254	0.84	480	0.92
	UCU	353	0.85	354	0.65	835	1.6*
	UCC	647	1.55*	114	0.38	459	0.88
	UCA	300	0.72	337	1.11	576	1.1
	UCG	376	0.90	345	1.13	296	0.57
Pro	CCU	459	0.84	586	1.21	730	1.44*
	CCC	581	1.06	325	0.7	278	0.55
	CCA	455	0.83	899	1.85*	707	1.40*
	CCG	701	1.28*	131	0.27	311	0.61
Glu	GAA	555	0.49	854	1.00*	1219	0.91*
	GAG	1724	1.51*	875	1.01*	1465	1.09*
Arg	CGU	231	0.66	178	0.98	433	1.22
	CGC	643	1.85*	153	0.84	152	0.43
	CGA	125	0.36	108	0.59	195	0.55
	CGG	314	0.90	51	0.28	139	0.39
	AGA	207	0.59	320	1.76*	663	1.87*
	AGG	569	1.63*	284	1.56*	544	1.54*
Thr	ACU	369	0.76	421	1.4*	827	1.39*
	ACC	777	1.61*	330	1.1	599	1.01
	ACA	328	0.68	351	1.17	645	1.08
	ACG	459	0.95	101	0.34	310	0.52
Lys	AAA	370	0.39	629	0.8	1043	0.78
	AAG	1540	1.61*	952	1.20*	1635	1.22*
Asp	GAU	645	0.68	716	1.22*	716	1.22*
	GAC	1240	1.31*	453	0.78	453	0.78
Ile	AUU	442	0.81	571	1.43*	998	1.24*
	AUC	1013	1.86*	353	0.88	1035	1.29*
	AUA	181	0.33	278	0.69	382	0.47
Gln	CAA	465	0.61	586	1.16*	763	0.98*
	CAG	1066	1.39*	422	0.84	789	1.02*
Phe	UUU	332	0.50	470	0.98*	682	0.79
	UUC	1006	1.5*	486	1.02*	1039	1.21*
Asn	AAU	353	0.55	497	0.91	719	0.82
	AAC	923	1.44*	599	1.09*	1043	1.18*
Tyr	UAU	236	0.45	421	0.94*	455	0.76
	UAC	808	1.55*	473	1.06*	745	1.24*
His	CAU	265	0.63	301	1.11*	413	1.00*
	CAC	572	1.37*	243	0.89	411	1.00*
Met	AUG	891	1.00	505	1.00	1106	1.00
Cys	UGU	151	0.49	133	0.81	332	1.04*
	UGC	470	1.51*	197	1.19*	307	0.96
Trp	UGG	420	1.00	262	1.00	471	1.00
TER	UGA	45	1.35*	23	0.99	43	1.15*
	UAA	23	0.69	31	1.29*	43	1.15*
	UAG	32	0.96	17	0.72	26	0.7

Summary of relative synonymous codon usage in three plant species. Codon usage was tabulated (N) for maize (100 genes), soybean (71 genes), and Arabidopsis *(112 genes). The relative synonymous codon usage (RSCU) is the observed frequency divided by the expected frequency assuming random codon usage. The most frequently used synonym for each amino acid of each plant is marked by an asterisk.*

Register Today ...

Early registration is encouraged to guarantee a place at the Third International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. The symposium will be held November 13-16, 1994, in Monterey, CA

Seven panels will convene to explore such questions as:

- Can small-scale results be extrapolated in assessing risk?
- Are there unique risks for Centers of Diversity?
- Can new viral pathogens be generated from transgenic plants?

Experiences in the commercialization of transgenic crop plants also will be discussed.

To receive a program announcement and a complete agenda, please fax your request to Office of Agricultural Biotechnology, (703) 235-4429.

Plant Genome Analysis by Single Arbitrary Primer Amplification

Peter M. Gresshoff
Plant Molecular Genetics
Center for Legume Research and Institute of Agriculture
The University of Tennessee
Knoxville, TN 37901-1071

Molecular genetics approaches have enriched the resolution of plant genome analysis. The ability to clone and sequence specific genome regions has added sequence-based information to our understanding of plant genomes derived from cytogenetics and large-scale DNA analyses (such as reassociation analysis).

While the database of DNA sequences is exponentially growing, methods are needed to investigate plant genomes at a level of complexity above the primary sequence, but below the cytogenetic, karyotypic arrangement.

Single, arbitrary primer-based DNA amplification techniques (DAF, RAPD and AP-PCR) were developed (Caetano-Anollés et al., 1991a; Williams et al., 1990; Welsh and McClelland, 1990), extending the utility of PCR to general genome analysis (fig. 1). Because of a plethora of terms, we proposed the general acronym MAAP (Multiple Arbitrary Amplicon Profiling; Caetano-Anollés et al., 1992b, 1993, 1994).

In essence, MAAP involves the use of a short, arbitrarily chosen oligonucleotide primer which, annealed to DNA, will direct DNA amplification of multiple genome regions (amplicons; Mullis, 1991). Temperature cycling and the use of a thermostable DNA polymerase are common components with the more specific and targeted PCR. In contrast to PCR, MAAP procedures use a single primer which is of arbitrary sequence. MAAP intentionally generates multiple products, which itself would be a rather undesirable result in a PCR reaction. MAAP is general, so that a primer used for one species can be used repeatedly for others, even if evolutionary distances between the template DNAs are large.

DNA amplification fingerprinting

identity testing/genetic profiling

molecular markers

pathogen identification

Figure 1: Uses of single primer amplification methods.

Amplification products are separated and recorded by a variety of detection methods; in all cases, a linear array of signals generates a profile, which is representative for the target DNA and specified by the DNA sequence of the primer. Variations in primer sites on the target DNA, length variations between primer sites, and possibly changes in the secondary structure of target DNA between or flanking the primer recognition sites, generate molecular polymorphisms. These amplification polymorphisms define molecular regions of the plant genome and thus can be used as (1) potential sequence tagged sites for positional cloning approaches, or (2) components of profile used in DNA profiling and diagnostics.

Abbreviations: PCR=polymerase chain reaction; *DAF*=DNA amplification fingerprinting; *AP-PCR*=arbitrary primer-PCR; *MAAP*=multiple arbitrary amplicon profiling; *nt*=nucleotide; *bp*=base pair; *PAGE*=polyacrylamide gel electrophoresis; *RAPD*=random amplified polymorphic DNA; *RFLP*=restriction fragment length polymorphism

Three Techniques

MAAP procedures were developed independently, and apparently concurrently, in three laboratories. Welsh and McClelland (1990) developed AP-PCR, which uses PCR-length primers [18 to 32 nt] of arbitrary sequence to amplify target DNA under low stringency annealing conditions for two amplification cycles. This allows abundant mismatching and the generation of multiple amplification products (equivalent to a PCR reaction having gone wrong). Increased stringency of annealing at later amplification cycles generated reproducible products which were resolved on polyacrylamide gels and detected by autoradiography.

Williams et al (1990) invented the RAPD procedure, in which an arbitrary primer of either 9 or 10 nt produced amplification products after temperature cycling. RAPD products are routinely resolved on agarose gels and visualized by ethidium bromide. This provides a rapid method of scanning a genome. Alternative methods of detection, such as PAGE and silver-staining, coupled with careful optimization of amplification parameters (Collins and Symons, 1993) improved the utility of the approach. RAPD is widely used because of its simplicity and low-cost instrumentation.

Caetano-Anollés et al. (1991a,b) developed DNA Amplification Fingerprinting (DAF). Of all MAAP procedures, DAF utilizes the shortest primers, down to 5 nt in length. The optimal length was found to be 8 nt, a length which does not produce efficient amplification with RAPD. Informative amplification profiles were generated with 5 nt primers (5-mers), using soybean DNA as a template (Caetano-Anollés et al., 1993).

DAF products are routinely separated by thin polyacrylamide gels, backed onto plastic Gel-Bond film. This gel-plastic support, which provides support during the washing steps and helps preserve the original gel, is stained by an improved silver-staining method (Bassam et al., 1991; Caetano-Anollés and Gresshoff, 1994a), which detects DNA at about 1 pg mm^{-2}. Resultant gels are air-dried and kept for permanent record and evaluation.

Developments

Pattern Detection

The PAGE/silver-staining technique provides a low-cost, high-throughput analytical method of DAF products. DAF products were also resolved by alternative methods. Agarose gels give clear resolution, but fewer products (Prabhu and Gresshoff, 1994). Fluorochrome labeled octamer primers were generated which then directed amplification of plant DNA (Caetano-Anollés et al., 1992a). The resultant amplification products were separated on an ABI Sequencer using Gene Scanner software. Single nucleotide resolution was obtained for lower sized amplification products. Tests using capillary electrophoresis have been promising (Dr. Patrick Williams, DNA Testing Laboratory, AFIP, Gaithersburg, MD; personal communication), providing separation of single samples in 30 minutes. In general, DAF generates scoreable polymorphisms in the molecular size range from 100 to 800 bp. Recently, we have used the pre-cast and automated PhastGel system (Pharmacia Inc.) to obtain profiles for pathogenic nematodes on soybean (Baum et al., 1994). Bands at higher molecular weight (up to 1500 bp) were scoreable; species and race-specific polymorphisms were detected. Denaturing gradient gel electrophoresis (DGGE) is another method which would help to distinguish polymorphic products of wheat (He et al., 1992).

Genetic Uses of DAF

The ability to detect molecular markers closely associated with genes of agricultural importance makes marker-based breeding an attractive proposition. The need for maintaining large plant populations through advanced breeding cycles can be reduced by detecting heterozygotes. MAAP markers converted through cloning, partial sequence analysis and specific PCR primer synthesis may provide SCARs (sequence characterized amplified regions), which are diagnostic for either a gene region in a plant or a pathogen. Figure 2 cartoons the utility of RFLPs and MAAP

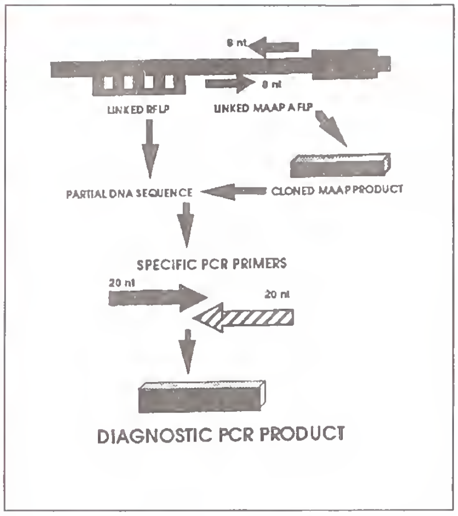

Figure 2: RFLP and MAAP markers used as diagnostic tools for genome analysis. Partial sequencing of clones, which are linked to your favorite gene (yfg!) provides information for specific PCR primers, which in turn generate a diagnostic product. A sequence characterized amplified region (SCAR) was demonstrated for the supernodulation gene of soybean by Kolchinsky et. al. (1994)

markers in generating diagnostic tools. For example, it may be possible to find markers specific for a soybean nematode race (see Baum et al., 1994), to convert it to a SCAR, then use a diagnostic, proactive test on agricultural soil to predict which nematode race is predominant in the field prior to planting.

The ability to generate many amplification products means that DAF is very efficient in scanning the genome of an organism for variable sites. In a survey of 25 primers (all octamers), Prabhu and Gresshoff (1994), working with *G. max* and *G. soja*, detected an average of 1.5 AFLPs per primer. Interestingly, RAPD gels of soybean produce an average of 5 to 7 scoreable bands, while DAF in soybean produced an average of 20 to 25 bands. Accordingly, the ratio of scored polymorphism to scoreable band is nearly the same, that DAF is not picking up more AFLPs because of the shorter primer length, but because of the detection method.

DAF markers were shown to be repeatable polymorphisms in different DNA isolations, operators, time periods, and amplifications. They are heritable, as are about 75% of AFLPs between *G. max* and *G. soja* segregated as dominant Mendelian markers in F2 populations (Prabhu and Gresshoff, 1994; Caetano-Anollés et al., 1993). Interestingly, the other 25% segregated in a uniparental way, being either maternal or paternal. Maternal inheritance presumably stems from amplification of cytoplasmic replicons. As yet, paternal replication is unexplained, and may represent either highly repeated chromosomal replicons or possibly alterations from normal cytoplasmic inheritance patterns in soybean.

Recombinant Inbred Lines

Several DAF polymorphisms were mapped in recombinant inbred lines of soybean (Prabhu and Gresshoff, 1994). The use of inbred lines is very convenient for DAF, as the lines are predominantly homozygous. Since DAF markers are dominant, it is impossible to distinguish the dominant homozygote from the heterozygote. Accordingly, in normal F2 populations, larger sample numbers are required to obtain data equivalent to data obtained from the analysis of a codominant (e.g., RFLP) marker. In recombinant inbreds, however, DAF and RFLP markers share the same statistical advantages. Figure 3 provides a summary of some RIL mapping data (conducted in collaboration with Dr. Gordon Lark, Utah).

The large number of products allows a high-density genotyping and genotype differentiation (Gresshoff, 1992). This form of fingerprinting is similar to the Universal Product Code, in which bars and spaces define a product. Reliable exclusion is obtained when one or more bands differ between samples. Inclusion is more difficult, as many primers need to be tested, frequency of variation within the sampled species needs to be known, and careful statistical statements need to be generated. One cannot

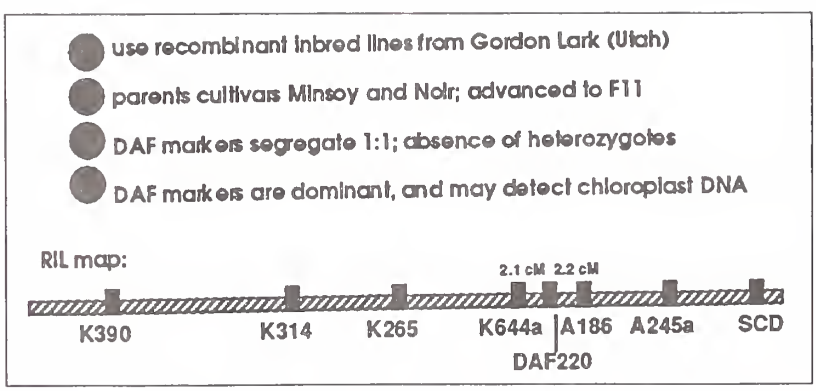

Figure 3: Summary of mapping of one DAF marker on recombinant inbred lines (F11) derived from a cross of soybean cultivars Minsoy and Noir.

declare with 100% certainty that two things are the same; the statement must always be probabilistic. It is up to the user (society, courts, scientists) to concur on acceptable levels of confidence for such probabilities.

Fingerprint Applications

DAF allowed the easy distinction of variant turfgrass material in commercial plots (Callahan et al., 1993). For example, foundation stock from several geographic locations gave identical profiles for Bermudagrass Tifway 419, while samples analyzed from golf course owners repeatedly showed major variation. The application of DNA tests to the turfgrass industry is a major challenge in an area of repeated vegetative propagation, triploidy and genetic instability. Using DAF markers, Weaver (1994) developed a phylogenetic tree of centipedegrasses.

Sunflower material provided by a seed company was categorized into several groups. Some common bands permitted the suggestion of a possible pedigree. This type of analysis has utility for product verification and plant variety rights.

The determination of genetic identity is also essential for the determination of plant product quality, as many food manufacturers use processes directly optimized for a specific biological feedstock. This industry relies on biological material; it is essential that quality biological feedstock enters the manufacturing process. Often it is impossible to inspect the source plant as one looks at a harvested product. It is for these industrial and related horticultural applications that a new technology was needed. DNA analysis has provided an additional way by which closely related organisms are distinguished for industrial., manufacturing, and retailing purposes.

DAF markers are useful in defining closely linked regions in bulked segregant analysis (Michelmore et al., 1991). The availability of large primer sets and the generation of multiple amplification products result in the efficient screening of the genome.

Induced plant mutations have the advantage of being in near-isogenic background as the genetic difference between parent and mutant is minimal. Using 25 DAF primers, Caetano-Anollés et al. (1993) showed that the induced supernodulation mutant nts382 and its wild-type parent cv. "Bragg" did not show polymorphisms despite the pairwise comparison of nearly 500 amplification products. Only in the use of MAAP, in which the target DNA was predigested with two restriction nucleases (four base cutters) and then amplified with a single octamer, could polymorphisms be detected between mutant and wild-type parent. Only 19 primers were needed to reveal 42 AFLPs. Fourteen of these segregated at 100% with the supernodulation phenotype in *G. soja* (wild-type) and *G. max* (mutant) derived F2 populations. Some AFLPs distinguished between the *nts382* and *nts1007* alleles. It is likely that these are valuable markers close to the *nts* locus and their cloning and further characterization will facilitate the isolation and ordering of yeast artificial chromosomes (YACs) in that region.

Mini-hairpin Primers

Funke and Kolchinsky (1994) demonstrated that stable YACs carrying soybean genomic DNA can be constructed, with an average size of about 200 kb (maximum 900 kb). About 7% represented chloroplastic DNA. The combination of clustered molecular markers, the ability to generate medium-sized YAC clones, end-clones and possible contigs, increase the chances of isolating soybean regions carrying developmentally significant genes. Caetano-Anollés and Gresshoff (1994b) used mini-hairpin primers in a DAF reaction to profile such soybean YACs. The mini-hairpin primers are interesting, because they contain on their 5' end a 7 nucleotide fold-back loop (4 nt in stem, 3 nt in the loop). The 3' end can be as short as 3 nt, allowing the generation of a small set of 64 primers, which are useful for the characterization especially of small genomes or genome components such as plasmids or YACs.

These findings show that single primer DNA amplification analysis of plant genomes adds a further genetic tool to construct high-density maps needed for positional cloning and marker-based breeding approaches.

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Primer length: 5 nt minimum; 8 nt optimum

Primer 3' end most important for specificity of reaction

Primer concentration: 3μM for 8-mer primer and up to 30μM for 5-mer primer

2mM $MgCl_2$ optimum for soybean genome and 6mM optimum for bacterial genome

Taq polymerase produces good amplification results for large fragments

Truncated Stoffel fragment should be used to amplify fragments in the 50-200 bp range

Excess template DNA (>25 ng/25 μl reaction) reduces intrinsic amplification products

For a more complete discussion of these parameters, please gopher to: ***gopher.nalusda.gov****. Select Information Centers from the menu. Next select Plant Genome Data and Information Center. If you would like a hard copy of the paper, please contact the Plant Genome Data and Information Center at the address on page 3.*

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Other Pursuits

Distinctive Biology of Forest Trees Highlighted at Sixth International Meeting

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Forest trees and their molecular genetics were the focus of a recent conference (Prouts Neck, Maine, USA, May 20-23, 1994) organized by Michael Greenwood and Keith Hutchison (University of Maine, USA). A group of 70 researchers gathered to discuss progress toward understanding and manipulating molecular processes within this diverse group of economically and ecologically important species.

The biology of forest trees provides both distinct challenges and unique opportunities. Compared to previous meetings of this research community, significant progress has been made in several areas. Research teams continue to isolate and characterize new genes, while transgenic plants, especially in *Populus*, are increasingly being used to study gene function *in vivo*. Genome mapping has also matured. In earlier meetings, presentations described the construction of genetic linkage maps, while at this meeting maps were presented as tools to identify and dissect quantitative trait loci (QTL).

Advantages of the haploid genetics offered by conifer gametophytes continue to be exploited for mapping work and for population surveys. Recent advances in model organisms continue to influence studies in forest trees. Homeotic genes, for example, are being sought for flower and cone development. Other research focuses on processes that either are unique to trees or are simply more important in trees than in other organisms. For example, lignin and its related biosynthetic pathways are affected by wounding and stress in trees as in other plants, while in trees alone, lignin also is a major component of wood.

Gene Expression Patterns

As highlighted by Olof Olsson (Swedish University of Agricultural Sciences, Sweden), woody angiosperms show fluctuations in gene expression during annual cycles of quiescence and "reesence" in addition to changes observed during development and in response to environmental stresses. With the goal of increasing pulp production efficiency, Wout Boerjan's laboratory (Universiteit Gent, Belgium) has produced transgenic *Populus* plants containing antisense constructs for the lignin biosynthetic enzymes O-methyltransferase (OMT) and cinnamyl alcohol dehydrogenase (CAD). Work on conifer lignin and its role in development continues despite being hindered by the lack of reliable transformation and regeneration methods. Working on *Pinus taeda*, John Mackay (North Carolina State Biotechnology Group, USA, directed by Ron Sederoff) described the isolation and characterization of several genes encoding lignin biosynthetic enzymes including phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and 4-coumarate:CoA ligase (4-CL).

A slightly different tack toward understanding xylogenesis is being taken by Mackay's colleague, Malcolm Campbell. Because myb-like genes play an important role in signal transduction pathways in other organisms, such genes may also control conifer xylem development. Campbell has initiated cloning of *Pinus taeda* homologues. A similar rationale underlies the strategy of Sharon Regan and Bob Rutledge (Petawawa National Forestry Institute, Canada) in their efforts to characterize MADS box homeotic genes controlling cone development

in *Picea mariana*.

With the goal of understanding the role of flavonoids in root formation, Lise Jouanin's laboratory (INRA, Versailles Cedex, France) has produced transgenic *Populus* and *Juglans* containing altered levels of chalcone synthase (CHS). Carmen Diaz-Sala, Keith Hutchison, and Mike Greenwood (University of Maine, USA) are investigating the cellular and molecular changes associated with the organization of root primordia in *Pinus taeda*. In particular, they are addressing how the cytoskeleton orients the plane of cellular divisions and nuclear reorganization.

Engineering for pest resistance using proteinase inhibitors is being done by several groups including those of Ned Klopfenstein (USDA-FS Center for Semiarid Agroforestry, USA), Lise Jouanin, and Seguin Armand (Universite Laval, Canada). Jouanin has found a cysteine protease inhibitor to be particularly effective against pests which contain high levels of cysteine proteases.

Genes responding to major environmental stresses have been identified in stress-specific cDNA libraries. Genes induced by drought stress in *Pinus taeda* (Shujun Chang et al., Texas A&M University, USA) include caffeoyl CoA, SAM synthetase, chitinase, and a protein similar to an animal skin matrix component. Genes induced by ozone (Dieter Ernst, Institut fur Biochemische Pflanzenpathologie, Germany) in *Pinus sylvestris* include CAD, stilbene synthase, hydroxymethylglutaryl-CoA-synthase, and polyubiquitin. As found in angiosperms, certain conifer genes appear to be induced by a number of environmental stresses. For example chitinase is induced by wounding, fungal infection, and drought (Haiguo Wu, Craig Echt, and John Davis, University of Florida, USA).

To expand upon the identification of new conifer genes, Claire Kinlaw (Institute of Forest Genetics, USA) has initiated "single-pass" sequencing efforts of *Pinus taeda* seedling cDNAs used as markers by David Neale and co-workers (Institute of Forest Genetics, USA) for genetic maps. These identified sequences will provide molecular tools for studying conifer genome organization and evolution. Early results have been encouraging in that a variety of genes have been identified including those encoding photosynthetic proteins, translation factors, glycolytic enzymes, and stress-response proteins.

With a systemic point of view, Gary Coleman (Oregon State University, USA) proposed a model to explain how trees regulate autumn nitrogen storage and spring remobilization in response to nitrogen availability and photoperiod. In this model, bark and leaves communicate with each other using a bark storage protein (BSP) and a leaf protein encoded by Win4. During short days or high levels of nitrogen, BSP accumulates in bark parenchyma while the Win4-encoded protein is repressed. During long day shoot growth, BSP is degraded while the Win4-encoded protein accumulates.

Genetic Maps and Quantitative Trait Loci

Genetic maps using two alternative approaches are being used to identify QTLs. Lively discussions of the merits and disadvantages of these two alternate approaches accompanied formal presentations. Mitch Sewell (Institute of Forest Genetics, USA) described the integration of restriction fragment length polymorphism (RFLP)-based linkage maps from two *Pinus taeda* pedigrees. This work will further efforts by Neale and his co-workers to dissect wood quality traits and to understand conifer genome organization and evolution. Several members of the Forest Biotechnology Group at North Carolina State University (USA) presented RAPD-based maps including those from *Eucalyptus* (Dario Grattapaglia) for the identification of QTL controlling sprouting and rooting.

New Markers

Several laboratories are exploring the use of length polymorphism among simple sequence repeats (SSRs). Craig Echt, (USDA Forest Service, Rhinelander, USA) has observed that approximately 0.7% of the *Pinus strobus* genome is comprised of SSRs. Of the primer pairs tested from the flanking sequences of *Pinus strobus* SSR loci, approximately 65% reliably amplify DNA. A high proportion show size polymorphisms, and a significant number amplify DNA from other conifer taxa. In apparent contrast to these observations, Keith Hutchison (University of Maine, USA) has observed a low level of size polymorphisms among SSR alleles in

Larix laricina.

With a similar goal of developing co-dominant PCR-based markers, but using a different approach, David Harry (Institute of Forest Genetics, USA) is designing and testing primers based upon sequences of specific *Pinus taeda* cDNAs. Approximately 75% of the primer pairs reliably amplify genomic DNA, with a high proportion revealing Mendelian polymorphisms following restriction enzyme digestion. Some primers amplify only hard pines, others amplify all pines, and still others amplify DNA from other conifer taxa. Hisato Okuizumi presented an application of restriction landmark genomic scanning (RLGS) to large genomes by including a restriction trapper. High-speed scanning of entire genomes and the construction of genetic maps of individual trees from a single run with several hundred loci are made possible. As an example, a profile of *Pinus koraiensis* was shown.

Describing Genome Flux and Evolution

Because seed plants represent an ancient lineage, and because woody plants have long generation times, mechanisms of genetic mutation and genome evolution, as well as rates of species evolution, continue to be important areas of study. In seeming contrast to low levels of observed SSR polymorphism in *Larix laricina*, Hutchison and coworkers have found relatively high levels of sequence variation within genomic regions encoding proteins.

In addition, they have observed segregation distortion of alleles under different environments. The apparent contrast between the high levels of polymorphism among coding regions and low levels of polymorphism among SSR regions may indicate that conifers have a relatively efficient mismatch repair mechanism and may thus partially account for the stability of conifer karyotypes.

Jean Bousquet (Universite Laval, Canada) and his co-workers are investigating ancient events during the evolution of seed plants. After carefully calibrating a molecular clock, they established that modern gymnosperms derived from a single lineage, and they estimated divergence times to have occurred as follows:

liverworts from vascular plants
440 mya
ferns from seed bearing plants
400 mya
flowering from cone bearing plants
290 mya
monocots from dicots
200 mya
Pinus from *Pseudotsuga*
140 mya

Ross Whetten (North Carolina State University, USA) is exploiting the idea that a tree's crown represents a common lineage of shoots with known separation times. Using visible phenotypes in peach, Whetten estimated the somatic excision rate of a transposable element. The rate is relatively higher than rates reported for annual species and varies among meristematic layers. This notion that different shoots within the crown of a tree can be genetically distinct might help explain how long-lived trees endure pathogens and insect pests with shorter generation times.

Genetic Diversity and Population Structure

In addition to their use in mapping, RAPDs have been used by a number of laboratories for estimating genetic diversity and describing population structure. Natalie Isabel (Universite Laval, Canada) compared estimates of genetic variation within and among populations of *Picea mariana* using RAPDs and allozymes. Results from these two types of markers were similar. Linda DeVerno (Petawawa National Forestry Institute, Canada) surveyed *Pinus resinosa* using 400 RAPD primers and found no polymorphism. Again this data supports earlier conclusions based upon allozymes.

1995 Meeting

The next tree molecular geneticists meeting will take place at Universiteit Gent, Belgium, in combination with the IUFRO Somatic Cell Genetics Working Party. Those wishing more information are encouraged to contact Wout Boerjan (Boerjan%research%RUG.genetica@genwet1.rug.ac.be).

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- **Application of RFLP and RAPD Technologies in Plant Breeding**. January 1994. Compiled by Andrew Kalinski.
- **Application of PCR Technology to Plant Breeding**. January 1994. Compiled by Andrew Kalinski.
- **Patents on Transgenic Plants**. February 1994. Compiled by Andrew Kalinski.
- **Soybean Genome Mapping**. January 1994. Compiled by Andrew Kalinski.
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- **Transgenic Maize**. February 1994. Compiled by Andrew Kalinski.
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Miscellaneous Publications:

Prepared by the Biotechnology Information Center staff.

- **AG/Biotechnology Electronic Information.**
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